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Mouse models of DNA Double Strand Break Repair and Neurological Disease

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Abstract

The repair of DNA damage is essential for the prevention of disease. The DNA double-strand break (DSB) is a particularly hazardous lesion. DNA DSBs activate a coordinated cellular response involving cell cycle checkpoint activation and repair of the DNA break, or alternatively apoptosis. In the nervous system the inability to respond to DNA DSBs may lead to neurodegenerative disease or brain tumors. Therefore, understanding the DNA DSB response mechanism in the nervous system is of high importance for developing new treatments for neurodegeneration and cancer. In this regard, the use of mouse models represents an important approach for advancing our understanding of the biology of the DNA damage response in the nervous system.

Keywords

DNA repair; double strand breaks; homologous recombination; non-homologous end-joining; neurodegeneration; nervous system

Introduction

The human brain contains on the order of one hundred billion neurons that support a broad range of functions throughout neural tissue [1]. A process critical for maintaining homeostasis in the brain is an effective response to DNA damage. A variety of diseases result from defective DNA repair and many of these show pronounced neuropathology [2–4]. In many cases the syndromes associated with DNA repair defects impact developmentally, as the formation of the nervous system strictly requires the ability to respond to DNA damage in proliferating and immature neural tissue. However, many neurodegenerative diseases linked to DNA repair deficiency show surprisingly select effects within the CNS, indicating that the consequences of DNA damage vary considerably depending on the nature of the DNA damage signaling pathways that are perturbed [5,6].

Because DNA repair deficiency can impact so substantially upon the nervous system, there is a need to produce model systems with which we can understand the pathways and etiological agents that are important for maintaining neural homeostasis. In this regard, the mouse offers an important resource for analysis of the effects of genotoxic stress during both

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the development and maintenance of the nervous system. Moreover, links are also emerging between DNA damage and significant neurological diseases associated with aging such as Alzheimer's disease [7,8]. Therefore, generation of model systems to understand the tissue-specific importance of DNA repair will also be valuable aids for refining therapeutic intervention of neurological disease. In the following we will emphasize the contribution mouse models have made towards understanding the biology of DNA repair in the nervous system.

Development of the nervous system

The nervous system presents a substantial challenge for delineating the requirements for DNA DSB repair, both embryonically and in the mature nervous system. In the mouse, the nervous system initiates its development onwards from around embryonic day 11 (E11) until three weeks after birth. Nervous system development follows a reiterated pattern of proliferation, differentiation and migration and finally maturation [9]. Proliferation occurs in the neuroepithelium (ventricular zone: VZ) that contains neural stem and progenitor cells. Post-mitotic cells migrate through the sub ventricular zone (SVZ) to their final programmed destination where they will undergo maturation. Detailed reviews are available that summarize many aspects of neural development [10–14].

Rapid proliferation drives development throughout the nervous system, and this process is linked to concomitant maturation of many diverse neural regions [15]. Although the bulk of cells in the nervous system are either neurons or glia, within these broad classes are a myriad of specialized cell types, which are characterized by specific functional roles they play to support the divergent functions of neural tissue. These cellular subgroups are characterized by their morphology, the neurotransmitters used for cellular communications and the connections they form with other cells. For example, the retina is a relatively small (but important) part of the nervous system which contains over 55 different cell types [16], and during development the spinal cord generates a diverse set of functionally different cell types [17]. This complexity is reiterated through out the nervous system, and the diversity in cell types potentially impacts the specific requirements for DNA repair pathways. An additional consideration for DNA repair in the nervous system is the contrasting requirement for this process during development and maturation, as the genesis of the complexity in the nervous system results from widespread zones of proliferation that ultimately generate the diversity in the nervous system. With regard to DNA DSBs, the repair pathways that are required will be a function of the proliferative state of the tissue. A major DSB repair pathway, homologous recombination (see later) requires a sister-chromatid for error-free repair, and therefore operates in replicating cells [18,19]. Thus, at this stage faulty DNA repair could lead to expansion of mutant progenitors, which could contribute to disease later in the mature nervous system. In non-replicating cells, which is the case for the mature nervous system DNA DSBs are repaired by an alternate pathway, non-homologous end-joining ([20]; see later). While the mature nervous system is largely non-replicating, there are nonetheless regions in which ongoing proliferation and neurogenesis occurs. However, this neurogenesis is very restricted and is confined to only the subventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus, and overall is a minor component of proliferation in the mature brain [21–24]. Additionally, under some circumstances, such as disease or brain injury, limited glial cell proliferation may occur in the mature nervous system [25–27]. Recent advances in mouse genetics have allowed significant progress in our understanding of DNA repair in the nervous system. Below, we will outline the basic schema for the repair of DNA DSBs and illustrate the utility mouse models have played in deciphering the role of the DNA damage response in a tissue-specific setting. The utility of mouse systems for studying the role of nucleotide excision repair (NER) in the nervous system is described elsewhere in this volume, in a contribution from Laura Niedernhofer.

DNA DSB repair and mouse brain development

DNA DSBs initiate a signaling cascade that leads to the repair and resolution of the break or apoptosis. In the developing nervous system, apoptosis seems to be a common outcome after DNA DSBs (Figure 1) and may be a preferred strategy, as cells can be easily replaced from the abundant progenitor population present during neural development [28]. Basic strategies for repair of DSBs involve either non-homologous end joining (NHEJ) or homologous recombination repair (HR). These two major repair pathways maintain the integrity of DNA after endogenous DSBs or those arising from exogenous agents. Inactivation of either NHEJ or HR results in marked defects in nervous system development [28]. HR is an error-free process that uses a sister chromatid as template DNA to achieve precise repair [18,19,29]. Homology-directed repair initially involves DSB processing via Mre11-Rad50-Nbs1 (MRN) in collaboration with CtIP to generate 3' single stranded DNA that becomes bound by Rad51 to facilitate homology search and strand invasion [30–32]. The Rad51 recombinase functions in concert with a series of other factors including the Rad51 paralogs (Xrcc2, Xrcc3, RAD51L1, RAD51L2, RAD51L3), Rad52, and Rad54 proteins to promote strand invasion and subsequent recombinational repair [18,19,33–35].

In contrast to HR, NHEJ modifies the two DNA ends so that they are compatible for direct ligation and is the predominant pathway for repairing DSBs in non-cycling mammalian cells [20,36,37]. NHEJ is an error-prone DSB repair mechanism involving the DNA-dependent protein kinase (DNA-PK) and the DNA ligase IV (Lig4), which together facilitate re-joining of broken non-compatible DNA ends. The DNA-PK complex contains Ku70 and Ku80 proteins, which recognize the break and mediate recruitment of the DNA-PK catalytic subunit (DNA-PKcs), a protein kinase required for efficient NHEJ [36–38]. Ligation of the DNA ends occurs via Lig4 in conjunction with its binding partner Xrcc4. An additional factor, Cernunnos/XLF, has been identified as a binding partner of the Lig4-Xrcc4 complex and appears to be necessary for efficient ligation via NHEJ [39,40]. Subsets of NHEJ may involve other factors such as Artemis [41,42]. The choice of repair pathway that becomes activated is linked to the cell cycle, with HR being available in S- and G2-phases [43].

All components of the general DSB response machinery are present in the developing brain, although some reports suggest decreased activity of repair proteins are associated with ageing [44]. Inactivation of DNA repair genes in the mouse has been done extensively, and most known key repair factors have been targeted in the mouse [45]. In many cases, the inactivation of these factors is incompatible with viability and results in embryonic lethality. Nonetheless, DNA repair defective mice have provided many important insights about the interconnections between this particular DNA repair pathway and neural development. A key feature of DSB repair pathways in the developing nervous system is the requirement for HR in the proliferative zones in the nervous system, while NHEJ is important in the differentiating SVZ [46], although there can be significant cooperativity between these pathways [47,48]. Therefore, the DNA damage response will be related to specific developmental stages, with a limited repertoire being available in mature differentiated cells.

The role of NHEJ during mouse brain development

The core NHEJ components have all been subject to gene targeting in the mouse, and extensive phenotypic analysis has been undertaken in the resultant mutant nervous system [46,49–53]. Notably, inactivation of either Lig4 or Xrcc4 results in an identical lethal phenotype around midgestation, while inactivation of the DNA-PK complex components results in viable mutant animals [54]. Artemis-null animals are also viable, and while Xlf targeted ES cells are hypersensitive to DNA DSBs, Xlf-null mice have not yet been reported [55–57]. These differences in viability illustrate the relative dispensability of the NHEJ core

components, and point to the critical requirement for Lig4 in preventing the accumulation of DSBs.

Disruption of either *Lig4* or *Xrcc4* in mouse leads to widespread neural apoptosis that occurs throughout the developing nervous system and is associated with regions containing immature postmitotic cells [49–51]. In the case of Lig4 (and presumably Xrcc4) there is no clear deficit in the mutant embryos until around E12, a time that coincides with regional differentiation in the nervous system, suggesting that NHEJ is relatively dispensable until neural differentiation commences [46]. This probably indicates that in the non-dividing neural cells active NHEJ is required for DNA DSB repair, and that other repair pathways cannot effectively substitute. The increased neural apoptosis after NHEJ loss has been suggested to involve oxygen metabolism that generates endogenous DSBs at a higher frequency than in other organs [58]. In *Xrcc4* or *Lig4*-null mice, neural apoptosis and lethality is rescued by coincident inactivation of p53 [50,59]. In both cases double null animals succumb to lymphoma or medulloblastoma [50,59–61]. The occurrence of medulloblastoma (a brain tumour originating in the cerebellum) after inactivation of NHEJ probably reflects the importance for DNA DSB repair in the developing cerebellum. This organ continues to undergo substantial postnatal neurogenesis, and during this period of cerebellar growth produces granule neurons that will become the most abundant neuronal population in the brain [12,60].

Consistent with the need for effective repair of DNA DSBs by NHEJ, individuals with mutations in *LIG4* exhibit immunodeficiency, developmental delay, growth retardation, and microcephaly, a disease that has been termed *LIG4* syndrome (Table 1) [62,63]. Since knockout mice lacking functional Lig4 are not viable, the mutations in the *LIG4* syndrome patients are hypomorphic alleles. In view of the neuronal apoptosis in Lig4-deficient mice, it is possible that *LIG4* syndrome patients also experience elevated neuronal apoptosis during development, which could underlie the reported microcephaly and developmental delay (Figure 2).

While Ku70/80 or DNA-PKcs-deficient mice are viable, there is increased apoptosis in the Ku-null nervous system, although not in that of DNA-PKcs-null mice [54]. The increased apoptosis present in Ku70 and Ku80-deficient embryos was localized to the developing spinal cord, cerebral cortex, midbrain and hindbrain [54]. Apoptosis has also been found in the embryonic retina in Ku-null animals [64]. It has been suggested that neuronal cells might be especially prone to apoptosis in response to DSBs perhaps as a means to eliminate damaged neurons during development [28]. The ability of the Ku null mice to form a relatively intact nervous system suggests that the level of DNA DSBs is below that of the Lig4-null mice, and that sufficient cell replacement occurred to maintain neural development. Notably, Ku deficiency was shown to rescue embryonic lethality in some Lig4-null animals [65]. While the reason for the viability of the small percentage of Lig4/Ku double null mutants is not certain, it has been suggested that in the absence of Ku the DNA-PKcs-Artemis nuclease complex did not load onto the DNA, and thus minimized DNA erosion in absence of Lig4. Presumably, this reduced DNA degradation did not effectively activate apoptosis and was sufficient to promote viability in some Lig4-null animals. Thus, normal development of the nervous system has a particular reliance on NHEJ.

The role of HR during mouse brain development

Inactivation of many central HR proteins leads to embryonic lethality around gastrulation [45]. This early lethality points to the well-described and critical importance of these factors during cellular proliferation to maintain genomic integrity. However, while in most cases this precludes in-depth analysis of their function during neural development, some limited examples do help to provide key insights about how HR interfaces with neural development.

Additionally, circumvention of this limitation has been afforded by the use of conditional gene ablation in which the spatiotemporal gene inactivation can be controlled [66].

Important HR factors, including *Xrcc2*, *Brca1* and *Brca2*, that have been inactivated in the mouse have provided important information about their role during neural development. Germline inactivation of *Xrcc2* led to embryonic lethality by early to mid-gestation associated with profound apoptosis [46,67]. While *Xrcc2* loss usually led to lethality around embryonic day 10, when little neural development had commenced, some embryos survived longer to stages when it was possible to perform detailed analysis of the developing nervous system. In these embryos, *Xrcc2* loss was characterized by extensive apoptosis throughout the VZ of developing nervous system, clearly showing *Xrcc2* loss only affected proliferating cells [46]. This suggested that HR is essential for the repair of DSBs in proliferating cells, and that NHEJ is not able to substitute for loss of HR cells. Similar to NHEJ mutants, loss of p53 was able to rescue the lethality associated with *Xrcc2* inactivation, and the resulting mice were highly cancer prone, and developed a wide spectrum of malignancy including brain tumors.

Inactivation of *Brca1* also resulted in very early lethality, although some *Brca1* mutant mice were obtained by inactivating exon 11 which allowed development until mid-gestation [68,69]. The majority of the mutant embryos exhibited neural tube developmental abnormalities such as spina bifida and anencephaly, and the neuroepithelium of *Brca1* deficient embryos exhibited increased apoptosis [68]. Similar to these findings, Xu *et al.*, demonstrated that lack of *Brca1* led to widespread apoptosis within the nervous system associated with developmental defects such as exencephaly [69]. Finally, similar to example of *Lig4* or *Xrcc4* deficiency, p53 heterozygosity rescued the phenotype suggesting that many of the defects in *Brca1* deficient embryos were p53 dependent [69].

Germline inactivation of *Brca2* also leads to very early embryonic lethality in the mouse precluding assessment of its contribution to neural function [70–72]. However, a recent study using conditional inactivation of *Brca2* throughout the nervous system bypassed embryonic lethality and showed that loss of *Brca2* led to microcephaly and impaired development of the cerebellum [73]. These neurodevelopmental defects were mainly associated with increased DNA damage-induced apoptosis in proliferative cells but also in early post-mitotic neurons. Inactivation of p53 contributed to a significant but incomplete rescue of this phenotype by preventing cell loss associated with DNA damage-induced apoptosis. Similar to the defects in NHEJ, simultaneous inactivation of p53 with *Brca2* loss led to rapid development of medulloblastoma [73]. The importance of *BRCA2* in the nervous system is also underscored Fanconi anemia (FA) which can result from disruption of any one of a number of proteins required for DNA crosslink repair, including *BRCA2* [74]. FA individuals that results from bi-allelic *BRCA2* inactivation manifest neurodevelopmental abnormalities, and are prone to brain tumors [75]. Thus, HR is an important DNA repair pathway that is required for neural homeostasis, and its perturbation can result in disease (Figure 2).

DSBs signaling and human neurological disorder

Coincident with DNA DSB repair is the activation of specific signaling events that serve to initiate cell cycle arrest to facilitate DNA repair. DNA DSBs induce rapid phosphorylation of the histone H2A variant H2AX, which facilitates the recruitment of numerous proteins including NBS1, MDC1 and 53BP1 to the vicinity of the break [76–78]. These events amplify DNA DSB signaling by the ataxia-telangiectasia mutated (ATM) kinase to modulate the activity of multiple target proteins involved in cell-cycle checkpoints and DNA repair pathways. ATM is a key protein kinase that functions at the apex of a signaling pathway that coordinates cell cycle arrest after DNA damage [79]. ATM-dependent phosphorylation of

cell-cycle checkpoint effectors such as p53, Chk2, SMC1, BRCA1, and NBS1 occurs to activate G1, intra-S and G2/M checkpoints. Detailed descriptions of ATM-signaling after DNA damage have recently been published [78,80–82]. Simultaneously with DNA damage induced foci formation of factors that activate cell-cycle checkpoints, DNA repair machinery also co-localizes to the DSB [83,84]. See also other contributions in this volume for detailed discussion of the link between ATM-signaling and neurological disease.

An ATM-related protein kinase, ataxia-telangiectasia and Rad3-related (ATR) also participates in DNA damage signaling. ATM and ATR are both critical for nervous system function and can phosphorylate many common substrates including p53, NBS1, BRCA1 and Chk1 in response to DNA damage [85,86]. Furthermore, in response to DNA DSBs there is an ATM-dependent requirement for ATR signaling, whereby in the absence of ATM activation of a Chk1-dependent cell cycle checkpoint after ionizing radiation is delayed [87–90]. Additionally, ATM and ATR act cooperatively and in collaboration with Mre11 during the initiation and regulation of replication, and function to halt these processes when DNA damage is detected [87,91,92]. A shared role for ATM and ATR in telomere maintenance and modulation of FANCD2 has also been demonstrated [93,94]. Although the targets of ATM (and ATR) have been well characterized, they may be more diverse than previously thought as recent studies demonstrate that around 700 proteins can be phosphorylated by these kinases in response to ionizing radiation [95]. However, these kinases have non-overlapping functions, as defects in each lead to distinct neurological diseases in humans (Figure 2); inactivation of ATM leads to the neurodegenerative syndrome ataxia-telangiectasia (A-T), while hypomorphic mutations of ATR can lead to ATR-Seckel syndrome (Table 1) [3,63,96,97]. In the mouse, inactivation of ATR is lethal very early during development, while mice lacking ATM are viable, and although tumor-prone can often have a normal lifespan [98,99].

Inactivation of murine *Atm* has provided important information about the tissue-specific function of this kinase. *Atm*-null mice are hypersensitive to radiation because of gut and hematopoietic sensitivity and are sterile and cancer prone, although they fail to recapitulate the overt neurodegeneration found in the human syndrome [98,100–102]. However, *Atm*-null neurons showed a striking resistance to apoptosis after DNA damage resulting from a lack of p53 activation [101]. The protective effect of *Atm* deficiency after DNA damage was associated with post-mitotic neurons but not proliferating neural cells [46,103]. Accordingly, ATM deficiency did not efficiently rescue the neurogenesis defects associated with the loss of HR proteins such as *Xrcc2* or *Brca2*, but did lead to substantial neural rescue after loss of the NHEJ factor, *Lig4* [46,53,103,104]. These data suggest that ATM functions as a genomic surveillance factor in the developing nervous system that monitors cells as they exit the cell cycle to activate apoptosis and eliminate cells that have incurred genomic damage.

Linked to ATM activation, the MRN complex is also critical for the DNA DSB response [105–107]. MRN is composed of *Mre11*, *Rad50* and *Nbs1* and is involved in DNA repair, DNA damage signaling, cell cycle checkpoints and telomere maintenance [108]. Hypomorphic mutations of MRN components lead to ataxia-telangiectasia like disorder (A-TLD) for *Mre11* and Nijmegen Breakage syndrome (NBS) for *NBS1* (Table 1) [108–111]. These syndromes exhibit neuropathology including progressive neurodegeneration in A-TLD and microcephaly in NBS. Inactivation of any of the MRN proteins in the mouse leads to early embryonic lethality [112–114]. Mice expressing truncated or mutated proteins as disease models have also been generated (*Nbs1* [115,116], *Mre11* [117] and *Rad50* [118]), although none of them fully recapitulated the human syndrome. In fact, while mouse models of DNA repair syndromes appear to mimic many of the human disease phenotypes, they often don't show defects in the nervous system to the same extent as humans. However, new approaches such as conditional gene inactivation are providing important new information

about the tissue-specific requirements of DNA repair factors. Recently, conditional inactivation of Nbs1 in the mouse central nervous system resulted in neurological aspects reflective of NBS in which profound microcephaly associated with ataxia was observed [119]. Loss of Nbs1 was particularly severe in the cerebellum, which presented with an absence of foliation and an ectopic localization of Purkinje cells. This phenotype was also associated with proliferation defects in progenitor cells and increased apoptosis in post-mitotic neurons, a phenomenon that required p53 signaling [119]. Together these data highlight the key role of Nbs1, and by association the MRN complex and its signaling partners, in the maintenance and protection of the central nervous system against DSBs during development.

Summary & Perspectives

A large number of different cell types integrate function in the nervous system, and this diversity presents a challenge for understanding the dynamics of the DNA damage response required for homeostasis in the brain. DNA repair defects can lead to neuropathology affecting diverse neural tissue resulting in debilitating neurodegeneration, microcephaly or brain tumors. Therapeutic approaches for treating DNA repair diseases will require a multifaceted understanding of the requirement for DNA damage signaling during all stages of neural development, including that of mature neural tissue. While all of the human syndromes discussed above result from defects during developmental, it is less clear if any aspect of the disease phenotype reflects a requirement for the respective gene products in the adult, and if effects at later stages are secondary to problems incurred during development.

A powerful approach to experimentally address these issues is the genetic manipulation of DNA repair factors in the mouse. Already, substantial knowledge has been gained following germline disruption of many DNA repair factors, although in many cases this leads to lethality, precluding a detailed neural analysis [45]. Presently, analyses involving tissue-specific inactivation of DNA repair have been particularly informative about specific biological requirements for DNA repair factors in this tissue. It is clear that as these studies progress, many of the outstanding questions regarding tissue requirements for DNA repair pathways will be answered.

While mouse models provide a unique biological perspective for tissue-specific effects of DNA damage, some limitations exist in modelling human disease in the mouse. This is particularly problematic in the nervous system, in which the mouse appears to be markedly resistant to DNA damage compared with humans. For example, *Atm*-null mice don't manifest the overt ataxia typical of human ATM loss and similarly hypomorphic mutation of *Mre11* that results in neurodegeneration and A-TLD in humans doesn't appear to lead to neurodegeneration in mice. However, as mouse models become more refined, current limitations will be overcome for example by using combinatorial gene inactivation approaches to mimic the neuropathology seen in human diseases, and will provide a clear view of the biology of DNA repair and how this process functions to prevent disease.

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Figure 1. DSB responses are linked to developmental stage in the central nervous system
 The nervous system undergoes differentiation that follows a recurring pattern of proliferation (neural stem cells and progenitors), differentiation (post-mitotic neurons) and finally maturation. The differentiation status of neural cells determines the response to DNA damage and the repair and effector kinase that are activated. Proliferating cells use HR while NHEJ is the main repair pathways used by non-dividing cells. ATM appears to function in recently postmitotic neural cells, while ATR is required by proliferating cells. However, the functional overlap between these related kinases has yet to be fully elucidated. Both proliferating cells and immature, post-mitotic neural cells are highly susceptible to DNA damage induced apoptosis and this process requires the p53-Puma signaling pathway. Functionally important DNA repair or DNA damage signaling pathways, including those involving ATM/ATR, and in the mature nervous system are yet to be clearly defined.

Figure 2. Defects in DNA damage signaling can lead to neurological disease
The DNA DSB response involves a variety of different factors that activate DNA repair or DNA signaling. DNA DSBs are repaired by either non-homologous end-joining (NHEJ) or homologous recombination (HR); each pathway involves distinct molecular machinery. DNA damage is detected by sensors that involve the MRN complex and signaling effectors such as ATM or ATR that activate down-stream signaling that functions to activate cell cycle checkpoints or elimination of the cell via activation of apoptosis. When these processes are disrupted human syndromes that feature neuropathology can occur. Genetic manipulation of the mouse is being used to mimic these DNA damage defective syndromes. Representative human syndromes are listed that result from defective DNA damage responses together with comparative phenotypes that occur in mouse models.

Table 1

DNA DSB repair diseases with neurological features in human.

Syndrome	Gene	Neurological features
Ataxia-Telangiectasia (A-T)	ATM	Ataxia, neurodegeneration
A-T like disorder (A-TLD)	MRE11	Ataxia, neurodegeneration
Seckel syndrome	ATR	Microcephaly
Nijmegen Breakage Syndrome (NBS)	NBS1	Microcephaly
NBS-like syndrome	RAD50	Microcephaly
LIG4 syndrome	DNA-LIGASEIV	Microcephaly
Fanconi Anemia	FANC genes	Microcephaly
Cernunnos syndrome	Cernunnos/XLF	Microcephaly