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DNA Damage Response in Peripheral Nervous System: Coping with Cancer Therapy-Induced DNA Lesions

Ella W Englander

Department of Surgery, University of Texas Medical Branch, Galveston, TX 77555

Abstract

In the absence of blood brain barrier (BBB) the DNA of peripheral nervous system (PNS) neurons is exposed to a broader spectrum of endogenous and exogenous threats compared to that of the central nervous system (CNS). Hence, while CNS and PNS neurons cope with many similar challenges inherent to their high oxygen consumption and vigorous metabolism, PNS neurons are also exposed to circulating toxins and inflammatory mediators due to relative permeability of PNS blood nerve barrier (BNB). Consequently, genomes of PNS neurons incur greater damage and the question awaiting investigation is whether specialized repair mechanisms for maintenance of DNA integrity have evolved to meet the additional needs of PNS neurons. Here, I review data showing how PNS neurons manage collateral DNA damage incurred in the course of different anti-cancer treatments designed to block DNA replication in proliferating tumor cells. Importantly, while PNS neurotoxicity and concomitant chemotherapy-induced peripheral neuropathy (CIPN) are among major dose limiting barriers in achieving therapy goals, CIPN is partially reversible during post-treatment nerve recovery. Clearly, cell recovery necessitates mobilization of the DNA damage response and underscores the need for systematic investigation of the scope of DNA repair capacities in the PNS to help predict post-treatment risks to recovering neurons.

Keywords

peripheral nervous system (PNS); dorsal root ganglion (DRG); blood brain barrier (BBB); blood nerve barrier (BNB); cisplatin cross-links, cytosine arabinoside (ara-C); ionizing radiation (IR); chemotherapy-induced peripheral neuropathy (CIPN); base excision repair (BER); nucleotide excision repair (NER)

1. Introduction

The DNA of peripheral nervous system (PNS) neurons is exposed to greater endogenous and exogenous threats compared to that of CNS neurons, due to lack of protection by BBB and skull. Ample evidence that links breaching of BBB with brain pathology, underscores the importance of BBB in maintaining CNS homeostasis and function [1, 2]. The blood nerve barrier of the PNS, on the other hand, is less restrictive and consequently less protective under normal and more so under pathological conditions [3–5]. PNS neurons are relatively

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Corresponding author: Ella W. Englander, PhD, Department of Surgery, University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555 phone: 409-772-8197; elenglan@utmb.edu.

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exposed to circulating compounds including metabolites, inflammatory molecules, environmental contaminants as well as mechanical nerve injuries, all of which have the potential to damage essential cellular components and trigger dysfunction of the PNS [4, 6].

Among major exogenous threats to PNS neurons are chemotherapy and radiation treatments, which while designed to block proliferation of cancer cells, impact also post mitotic neurons. Although in theory antimitotic treatments should not be toxic to non proliferating cells and the DNA of PNS neurons should be spared, it is not. In fact, chemotherapy induced nerve damage and peripheral neuropathies create significant barriers to effective use of cancer combating treatments [7–9]. Despite critical importance to human health, to date very few studies have addressed the mechanisms of damage formation, DNA damage response (DDR) and repair capacity in PNS neurons. While it is generally assumed that DNA repair processes in the CNS and PNS are fundamentally similar, in view of physiological dichotomy between the two nervous systems, specialized differences are plausible. Here, I review emerging data that afford insights into DNA damage response (DDR) mechanism in peripheral nervous system.

1.1 DNA repair pathways in Central Nervous System neurons

Hundreds of proteins are involved in surveillance and repair of DNA damage in mammalian cells. These proteins are shared among the different repair pathways and cooperate in detection and repair of diverse types of DNA lesions. Since various organs and tissues encounter very different insults which in turn produce diverse types and severities of genomic damage, cells differ in their capacities for sensing, responding and handling specific DNA lesions [10]. The major mammalian DNA repair mechanisms include, the base excision repair (BER) process for handling base modifications and single strand breaks (SSRBs), mismatch repair process for replacing misincorporated/mismatched bases (MMR), nucleotide excision repair (NER) for removal of bulky lesions such as UV adducts and intrastrand cisplatin cross-links, proteins associated with Fanconi anemia phenotype, which remove interstrand cross-links (ICLs), while double strand breaks (DSBs) are repaired by homologous recombination or by the non-homologous end joining process [11–16]. Although the different repair pathways cooperate and share components to accomplish efficient and accurate DNA repair, cells and tissues differ in their capacity to resolve the different types of damage. For example, the rapidly proliferating intestinal mucosa cells have a robust MMR pathway [17], while human skin cells have an efficient NER pathway for removal of UV cross-links [18]. To counteract continuous generation of endogenous DNA damage associated with high metabolic rates, post mitotic neurons possess substantial capacity for repair of oxidative DNA damage, the salient byproduct of high oxygen consumption [19]. The major mammalian process for repair of oxidative DNA lesions is the base excision repair (BER) pathway, which is indispensable for all mammalian cells and absolutely critical in long-lived postmitotic neurons in the CNS [20]. In fact, loss of key BER enzymes is embryonic lethal [21], while mutations which affect proteins involved in processing of DNA ends, including tyrosyl-DNA phosphodiesterase 1 (TDP1) and aprataxin (APTX) result in neurologic deficits and neurodegeneration [22–24], similarly to defects in various genes of the NER pathway and double strand breaks repair processes associated with severe neurodevelopmental deficits and microcephaly [25, 26].

Although oxidative DNA lesions include primarily base modifications and single strand breaks, the spectrum of oxidative adducts is complex and in many cases requires specialized processing of damaged DNA ends. Typically, processing of DNA ends cannot be fully executed by the short patch BER sub-pathway and requires involvement of the more complex, long-patch sub-pathway of BER, which relies in part on proteins involved in DNA replication [27]. Notwithstanding, in view of the absence of DNA replication machinery in neurons, it is plausible that instead specialized repair mechanisms have evolved for mending

adducts that require the long-patch BER sub-pathway [28]. While BER is a major DNA repair process in the adult brain tailored to specific needs of the CNS, evidence for the importance of other repair activities and pathways also exists [29–31]. Mouse models involving lack of such repair activities have been linked to major neurodevelopmental deficiencies, but characterization of deficits which are uniquely manifested in the adult brain is still limited [32]. The issue is even more complicated, as differences in repair capacities among different regions of the adult brain have been observed and linked regional susceptibilities to specific insults, which subsequently trigger development of pathologic conditions [33, 34].

1.2 DNA repair in Peripheral Nervous System neurons

In contrast to considerable information on DNA repair processes in the CNS, information on DNA repair activities in peripheral nerve cells is scarce. While plausibly postmitotic neurons in both systems have quite similar DNA repair capabilities, specific data are still limited. An interesting question that awaits investigation is whether challenges posed to genomes of PNS neurons because of the relatively leaky blood nerve barrier (BNB), are counteracted by specialized repair processes which might have evolved to aid sustain neuronal longevity and function in the PNS. Conceivably, such needs arise in settings of various disease states and environmental exposures, which trigger systemic inflammatory responses that generate excessive oxidative stress damaging cellular components, including DNA (Fig.1). Interestingly, a recent report has linked reduced expression of the ERCC1-XPF nuclease, which is involved in several DNA repair processes, including nucleotide excision repair, interstrand cross-link repair and homologous recombination, with age associated peripheral neuropathy in a mouse model of progeria [35, 36], supporting the notion that unresolved DNA damage contributes to the development of peripheral neuropathies.

2. Peripheral Nervous System Responses to Antimitotic Therapies that Target DNA

2.1 Repair of cisplatin-induced cross-links

A serious threat to PNS neurons is posed by antimitotic therapies that target DNA. Commonly used regimen include old and new compounds of cisplatin, which form intrastrand cross-links designed to block DNA replication and transcription, and thereby inhibit proliferation of tumor cells. In addition however, cisplatin cross-links compromise DNA integrity and RNA transcription in differentiated cells and consequently undermine cellular function. In mammalian cells removal of cisplatin-induced cross-links is carried out by the NER pathway. Since NER relies in part on proteins involved in DNA replication, it is less effective in adult CNS and most likely limited also in PNS neurons. Hence, cisplatin-compromised DNA integrity is predicted to contribute to neuronal dysfunction in the PNS. In fact, peripheral nerve neuropathies are among the dose limiting factors in chemotherapeutic regimen of the various cisplatin compounds [9, 37].

Importantly, in contrast to only limited presence in the CNS following treatment, cisplatin was found to accumulate in rat [38] and human dorsal root ganglia (DRG) [39]. DRGs are supplied by fenestrated capillaries that render them more accessible to circulating compounds [40]. Interestingly, following treatment nucleolar abnormalities in DRG neurons have been reported, linking cisplatin induced deficits with disruption of nucleolar transcription of ribosomal DNA and subsequent deleterious reduction in protein synthesis [41]. This is consistent with the possibility that cisplatin induced cross-links specifically hinder the intense synthesis of ribosomal RNA, which is indispensable for function of the highly metabolic DRG neurons. Internalized cisplatin is also likely to produce persisting problems, as suggested in an earlier rat study, which detected significant accumulation of

cisplatin in DRGs and observed that DRG neurons go through a futile attempt to enter the cell cycle and subsequently undergo p53-mediated apoptosis [42]. Notably, this path was preventable by supplementation of nerve growth factor, which presumably reinforces the terminally differentiated state. Deleterious re-entry into cell cycle of CNS neurons in response to severe insults has also been reported [43]. If confirmed this would suggest fundamental similarities in DNA damage responses in CNS and PNS neurons under conditions of excessive DNA damage.

Importantly, a direct relationship has been established recently, between persisting levels of cisplatin-induced cross-links in DRG neurons and the degree of peripheral neuropathy, linking CIPN with the capacity for timely removal of cisplatin cross-links from DNA of PNS neurons [37]. Using cisplatin cross-link specific antibodies [44], Dzagnidze et al., [37] compared formation and removal of cross-links in DRGs and in the spinal cord (which is protected by BBB) of wild-type mice, and mouse models lacking the core NER proteins Xpa or Xpc. Accelerated accumulation of cisplatin cross-links in DRG neurons and earlier onset of sensory nervous system deficits were observed in NER deficient mice, implicating the NER process in resolution of cisplatin cross-links in DRG neurons. Interestingly however, about 30% of cisplatin cross-links were removed also in Xpa deficient mice, suggesting that in PNS other DNA repair processes also contribute to resolution of these adducts. Notwithstanding, even in the wild-type mice, about 30% of cisplatin adducts were refractory to repair and persisted in DRG neurons for days after drug administration. In further support for NER involvement in resolution of cisplatin cross-links in the PNS, Gurthie et al., [45] reported stimulation of transcription and translocation of the NER proteins, Xpa and Xpc from cytoplasm to nucleus in spiral ganglion neurons at the base coil of the cochlea, in response to administration of cisplatin. Interestingly, translocation was most pronounced in the apex of the spiral ganglion, suggesting differential regional susceptibilities to cisplatin-induced DNA damage in the PNS.

Resolution of cisplatin cross-links in PNS was also investigated in the context of BER. Studying the central BER enzyme, apurinic/apyrimidinic endonuclease 1 (Ape1), Jiang et al., [46] dissected the Ape1 DNA repair and redox functions and found that while the repair function of Ape1 augments survival of DRG neurons in the face of cisplatin exposure, the redox function of Ape1, does not. This was further linked to p53 and Gadd45 signaling pathways, consistent with involvement of both, BER and NER pathways in response to cisplatin damage in DRG. Similar dependence of DRG neurons survival on the repair function of Ape1 was observed also in the case of H₂O₂ challenge [47]. Most recently however, adverse effects of cisplatin in DRG neurons were mapped to mitochondrial DNA (mtDNA) damage. Podratz et al., [48] reported that in DRG neurons cisplatin binds to mtDNA, and inhibits both mtDNA replication and transcription leading to mitochondrial dysfunction and development of DRG injury. Importantly, within the six-day recovery time frame of the study, no evidence for repair of mtDNA, i.e., no removal of cisplatin adducts was observed, in agreement with the originally documented lack of effective NER pathway in mitochondria [49, 50]. Interestingly, however, recent evidence suggests that the mitochondrial DNA polymerase gamma (pol- γ) has the ability to replicate past UV-induced cyclobutane thymine dimers [51]. In the context of this new observation, the question arises if similar bypass replication activity by pol- γ might be relevant also to cisplatin cross-links.

2.2 Cytosine arabinoside (ara-C)-induced DNA chain termination

A different common chemotherapeutic compound designed to block DNA replication is cytosine arabinoside (ara-C), an analog of cytosine which slows DNA synthesis and blocks DNA ligation. Ara-C is an 'old' antineoplastic drug used for nearly five decades for treatment of hematopoietic cancers, primarily leukemia. Ara-C is referred to as 'chain terminator' with dose limitations dictated largely by its neurotoxicity, neurologic deficits

and peripheral neuropathies [52, 53]. In addition to peripheral neuropathies severe cerebellar toxicity, has also been reported [52], suggesting significant extent of BBB crossing. In a rat model, observed adverse manifestations include cerebellar atrophy, loss of Purkinje cells and reactive proliferation of glia and astrocytes [54]. Although neurotoxic effects of ara-C have been reproduced *in vitro* in primary neuronal cultures [55], the mechanism of ara-C action remains not well understood, since in neurons ara-C incorporation into DNA is predicted to be limited to the extent of DNA repair synthesis. Inhibition of UV-induced DNA repair synthesis by ara-C was observed early on in proliferating cells [56]. Interestingly, Geller et al., [57] reported induction of single strand breaks in genomic DNA following exposure of primary cortical neurons to ara-C, but because neuronal death was attenuated in the presence of antioxidants and ara-C incorporation into nuclear DNA was only marginal, the authors concluded that the impact on DNA was indirect and mediated by oxidative stress. Important insights into ara-C mechanisms of action come, however, from earlier work in non-neuronal cells. Heintz and Hamlin [58] reported that even in replicating cells, a substantial fraction of incorporated ara-C becomes associated with mitochondrial DNA, whose synthesis is also sensitive to the inhibitory action of ara-C. This observation implicating compromise of mitochondrial DNA, affords critical insights into the mechanism of ara-C toxicity in post mitotic neurons. Interestingly, a recent study suggested that nuclear DNA polymerases indeed may vary in their ability to incorporate ara-C and other nucleoside analogs. The study reported that DNA polymerase lambda (pol- λ) incorporates ara-C relatively efficiently into DNA and thereby may effectively increase cytotoxicity of this drug [59]. DNA pol- λ is a member of the X family of DNA polymerases, involved in handling oxidative DNA damage, and shares properties with DNA polymerase beta (pol- β), the major BER polymerase. Studies with pol- β deficient mouse embryonic fibroblasts revealed that DNA pol- λ can substitute for pol- β in BER process [60]. Whether DNA pol- λ is present and active in the CNS and/or DRG neurons and thereby renders ara-C more neurotoxic in this setting remains to be determined.

A related question arises also in the context of the Y-family of specialized, low fidelity replication polymerases, which catalyze DNA synthesis across sites of DNA damage [61–63]. Since recently, the structural basis for translesion synthesis across cisplatin cross-links was revealed for the Y-family polymerase eta (pol- η) [64], the pressing question is whether specialized processes which uniquely facilitate transcription across adducts in genomic DNA, or replication across adducts in mitochondrial DNA might have evolved in CNS or PNS neurons.

2.3 Repair of ionizing radiation-induced DNA damage

A recent study examined manifestations of ionizing radiation in PNS neurons [65]. The study found that ionizing radiation (IR) leads to formation of transient as well as persisting DNA adducts. While the hallmark of ionizing radiation is the double-strand break (DSB), extensive oxidative DNA damage is also formed [66]. DSBs are the most genotoxic DNA adducts and pose particular threat to post-mitotic neurons, which rely on the error prone non-homologous end joining (NHEJ) process for their resolution. NHEJ repair of DSB is not templated and associated with formation of deletions and insertions that compromise genomic stability and are cytotoxic. Interestingly, Casafont et al. [65], observed inhibition of transcription shortly after IR, with subsequent recovery and progression of DSB repair within one day of exposure. Nonetheless, as judged by remaining γ H2Ax foci, residual DNA damage persisted in compartmentalized chromatin domains. Ionizing radiation-induced DNA adducts are not limited to DSBs and oxidative DNA damage is also substantial [66]. In further support of the possibility that BER process is involved in resolution of ionizing radiation damage, a recent study demonstrated that the repair function

of Ape1, the central BER enzyme, mediates protection of DRG neurons from ionizing radiation [67].

3. Detection and Localization of DNA Repair Proteins in Dorsal Root Ganglion (DRG) Neurons

To assess the scope, levels and distribution of DNA repair proteins in the PNS, we examined cultured rat primary DRG neurons for expression of key proteins involved in BER and NER repair processes. Immunofluorescent staining analyses (Fig 2) revealed substantial nuclear expression of major BER proteins including, apurinic/apyrimidinic endonuclease 1 (Ape1), DNA polymerase β (pol- β), DNA ligase 3, x-ray cross complementation group 1 (Xrcc1), flap endonuclease 1 (Fen-1), the NER protein xeroderma pigmentosum group a (Xpa), as well as robust expression of the mitochondrial DNA polymerase γ (pol- γ), consistent with the high metabolic demands and high oxygen consumption of DRG neurons. Although preliminary, these findings are in agreement with emerging data suggesting that the major DNA repair pathways, BER and NER are present and involved in DNA damage responses in PNS neurons.

4. Perspective

Peripheral nerve impairments incidental to disease states and cancer therapies pose significant clinical challenges. To facilitate the development of interventional ameliorative strategies, exact mechanisms of injury must first be identified. While undoubtedly mechanisms of PNS injury and resultant peripheral nerve neuropathies involve a broad range of physiological systems, a better understanding of fundamental mechanisms, which underlie the formation of DNA damage and limitations of the DNA damage response in the PNS, is critical. Because of neuronal longevity and vigorous metabolism, meticulous post-injury restoration of genomic integrity is vital for continued neuronal function. Studies are urgently required to elucidate the DNA damage response mechanisms in PNS neurons and thereby help improve risk assessment and prognosis.

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Figure 1. Scope of endogenous and exogenous insults that adversely impact PNS neurons: Types of DNA lesions and inferred range of DNA Damage Responses.

Figure 2.

Expression of DNA repair proteins in DRG neurons. Double fluorescent staining images of cultured DRG neurons reacted with antibodies for BER and NER proteins are shown. DRG neurons are identified by immunoreactivity for neurofilament 200 (NF200). NF200 staining reveals the oval shape of DRG neuron soma and neuronal extensions (red, top), whereas nuclei in all images are identified by Ape1 (apurinic/apyrimidinic endonuclease 1) immunoreactivity (green, center panel). Red nuclear staining (left panel) detects the indicated DNA repair proteins: BER pathway DNA polymerase beta (pol- β), DNA ligase 3, scaffold protein Xrcc1, the long-patch BER 5'-flap endonuclease 1 (Fen-1) and the core NER protein xeroderma pigmentosum group A (Xpa), whereas mitochondrial DNA

polymerase gamma (pol- γ) immunoreactivity shows the expected extra-nuclear localization (red, bottom). Images merged with DAPI staining (cell nuclei, blue) are shown in the right column (merged). In some images prominent nucleolus can also be observed. Scale bar=25 μ m. Images were captured using IX71 Olympus fluorescence microscope with QIC-F-M-12-C camera. Cultures were prepared from rat lumbar DRGs.