Breaks in Coordination: DNA Repair in Inherited Ataxia

Minireview

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Genetic defects in DNA repair are increasingly recognized as being able to cause degenerative ataxia syndromes. It remains a mystery, however, why disruption of a process fundamental to proliferating cells can be selectively toxic to postmitotic neurons. Recent studies now reveal that an ataxia gene, tyrosyl phosphodiesterase 1 (*TDP1*), repairs single-stranded DNA breaks in nondividing cells. Here we review the implications of this and other findings for a growing list of hereditary ataxias.

Why, in so many neurodegenerative diseases, do discrete populations of neurons die despite widespread expression of the disease protein? Take, for example, the growing list of degenerative ataxias now known to be caused by defects in DNA repair. In these ataxias, disruption of a process fundamental to all cells seems to hit neurons especially hard. Indeed, in some of these disorders, such as spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), the defect in DNA repair seems to affect *only* neurons. A recent report now provides important clues to pathogenic mechanisms in SCAN1 and other ataxias caused by defects in DNA repair (El-Khamisy et al., 2005). This and other studies (e.g., Frappart et al. 2005) are beginning to shed light on how different kinds of DNA damage result in toxicity to the nervous system, in some cases quite selectively.

First, a little background on the genetic basis of degenerative ataxias is needed. Ataxia means, quite literally, the loss of coordination, particularly of gait and limb. It most often results from degeneration of the cerebellum and its associated pathways. But damage to nearly any region of the neuroaxis can manifest with gait ataxia, thus it is not surprising that a remarkably wide spectrum of gene defects can cause ataxia (Taroni and DiDonato, 2004). For example, more than 25 genetic loci have been identified for the dominantly inherited ataxias alone. While the number of primary recessive ataxias lags behind, a great many recessively inherited metabolic disorders also cause ataxia as part of a broader clinical syndrome. Dominant ataxias tend to manifest in adult life, while recessive ataxias begin in early to late childhood.

The complexity of inherited ataxias notwithstanding, a few common themes of pathogenesis are emerging. For example, many of the better defined dominant ataxias are caused by expansions of polyglutamine tracts that confer a novel toxic property on the respective disease protein (Ross and Poirier, 2004). In con-

trast, the most common recessive ataxia, Friedreich ataxia (FA), is due to the loss of a nuclear-encoded mitochondrial protein, frataxin, which plays an essential role in mitochondrial iron homeostasis and the biosynthesis of iron-sulfur cluster-containing enzymes (Pandolfo, 2003). Mounting evidence suggests that oxidative stress is a primary mediator of pathogenesis in FA and several other ataxias, including ataxia with vitamin E deficiency and certain mitochondrial disorders.

It is increasingly clear that defects in DNA repair are yet another common cause of recessive ataxia (Table 1). The first such ataxia to be identified, ataxia telangiectasia (AT), is characterized by early onset, progressive ataxia with cerebellar atrophy, ocular and skin telangiectasias, immunodeficiency, and malignancies (McKinnon, 2004, and references therein). AT is caused by mutations in the protein kinase ATM, the master regulator of the cellular response to double-stranded DNA breaks (DSBs). Similar ataxic syndromes result from other defects in DSB repair. The rare AT-like disorder, so named because of its close resemblance to AT, is caused by mutations in *MRE11* (Stewart et al., 1999). Mre11 is one of three proteins comprising the Mre11/Rad50/Nbs1 (MRN) complex that senses DSBs and recruits and activates ATM at these sites of DNA damage (Lee and Paull, 2005, and references therein). Defects in a second protein of the MRN complex, Nbs1 or nibrin, underlie Nijmegen breakage syndrome, a developmental disorder that shares many features with AT, although the brain impairment occurs earlier and is more widespread (Digweed and Sperling, 2004).

In general, defects in DSB repair do not affect the nervous system exclusively. They also lead to adverse, systemic effects of genomic instability, including immunodeficiency and malignancy. In contrast, the recently discovered ataxias due to defects in single-stranded DNA repair appear to be confined to the nervous system outside of a few mild lab test abnormalities. They also show remarkable clinical and pathological similarity. Ataxia with oculomotor apraxia type 1 (AOA1), which is caused by mutations in a DNA repair protein, aprataxin, manifests with early onset ataxia, sensorimotor neuropathy, and a failure in eye movements, yet has no features outside of the nervous system (Le Ber et al., 2003, and references therein). The clinically very similar AOA type 2 is caused by mutations in senataxin, which contains a DNA/RNA helicase domain and likely functions in RNA processing and transcription-coupled DNA repair (Moreira et al., 2004). Interestingly, mutations in senataxin also cause a juvenile form of amyotrophic lateral sclerosis (Chen et al. 2004). And SCAN1, which has been shown to be due to an inactivating mutation in the topoisomerase I (Topo 1)- dependent DNA damage repair enzyme tyrosyl phosphodiesterase 1 (Tdp1), is characterized by ataxia and severe sensorimotor neuropathy without systemic features (Takashima et al., 2002).

Tdp1 is the focus of the study by El-Khamisy et al. (2005). Tdp1 functions in DNA repair by removing Topo1 that is covalently bound to DNA in stalled complexes

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Table 1. Recessive Ataxias Due to Defects in DNA Repair

Disease	Gene	Function	Neurological Findings	Non-Neurological Findings
Double-Strand Breaks				
Ataxia telangiectasia (A-T)	ATM	regulates cellular response to DSBs	ataxia, OA, choreathetosis, neuropathy	telangiectasia, immunodeficiency, cancer predisposition
Ataxia telangiectasia-like disease (ATLD)	MRE11	component of MRN complex	ataxia, OA, choreathetosis, neuropathy ch	telangiectasia (+/−), immunodefiency, cancer predisposition
Nijmegen breakage syndrome (NBS)	NBS1	component of MRN complex	microcephaly, MR	growth retardation, immunodeficiency, cancer predisposition
Single-Strand Breaks				
Ataxia with oculomotor apraxia 1 (AOA1)	APTX	interacts with SSB repair scaffold protein, XRCC1; nucleotidyl-lysine hydrolase	ataxia, OA, neuropathy	none
Ataxia with oculomotor apraxia 2 (AOA2)	SETX	helicase domain-containing protein; may function in RNA processing and transcription-coupled NER	ataxia, OA, neuropathy	none
Spinocerebellar ataxia with axonal neuropathy (SCAN1)	TDP1	tyrosyl phosphodiesterase	ataxia, neuropathy	none
Xeroderma pigmentosum (XP)	8 Loci	genome and transcription-coupled NER	In ~30% of patients: microcephaly, hearing loss, MR, neuropathy	extreme photosensitivity, ocular and skin cancers
Cockayne syndrome (CS)	CKN1, ERCC6	transcription-coupled NER	ataxia, MR, spasticity, hearing and vision loss	growth failure, dental caries, photosensitivity

Mutations affecting DSB repair manifest with neurological and systemic features while mutations affecting SSB repair tend to be confined to the nervous system. Minor laboratory abnormalities have been observed in AOA1, AOA2, and SCAN1: hypercholesterolemia (AOA1, AOA2, SCAN1), hypoalbuminemia (AOA1, SCAN1), and elevated serum alpha-fetoprotein (AOA2). NBS, XP, and CS represent syndromes with widespread neurological phenotypes but not primarily ataxia. Oculomotor apraxia, OA; mental retardation, MR; nucleotide excision repair, NER.

(Connelly and Leach, 2004). Such DNA-Topo1 adducts are a natural consequence of the biological action of Topo1: Topo1 unwinds double-stranded DNA by cleaving one DNA strand, in the process forming a 3′phosphotyrosine intermediate. The intact DNA phosphodiester backbone is usually restored spontaneously, but Tdp1 provides an additional measure of genomic surveillance to enzymatically remove DNA-Topo1 adducts that accumulate upon DNA damage. Through its action, Tdp1 helps to prevent DSBs that occur when replication forks encounter stalled complexes during DNA replication.

Due to redundancy in DSB repair, however, loss of Tdp1 function results in little or no observable phenotype in replicating tissues. So how does loss of Tdp1 cause a dramatic phenotype in nonreplicating neurons? To address this question, El-Khamisy et al. (2005) mimicked the postmitotic state of neurons by arresting mitosis in lymphoblastoid cells from SCAN1 patients and then exposed the cells to the Topo1 poison, campto-thecin A. Camptothecin treatment caused abundant breaks in the DNA, which turned out to be almost exclusively single-strand breaks (SSBs). When camptothecin was removed, SCAN1 cells did not repair the breaks, whereas wild-type cells did so rapidly. Importantly, SSBs induced by a second DNA damaging agent, oxidative stress, also were inefficiently repaired in SCAN1 cells. Additional yeast two-hybrid, coimmunoprecipation, and reconstitution studies showed that Tdp1 associates with the SSB repair machinery and

likely functions as an essential component within this multiprotein complex. In short, a protein already implicated in DSB repair in replicating cells and in SSB repair in yeast (Plo et al., 2003) has now been found to function in a novel SSB repair pathway in nondividing human cells. Independently, Zhou et al. (2005) have also shown that mutant Tdp1 no longer removes 3′ DNA phosphoglycolates formed by free radical-mediated DNA cleavage.

How might accumulated SSBs affect postmitotic cells? One possibility is that they perturb transcription. El-Khamisy and colleagues tested this by inducing SSBs with camptothecin, which acutely reduces transcription, then measuring transcriptional recovery over time in wild-type versus SCAN1 lymphoblastoid cells. Unlike control cells, which rapidly recovered, SCAN1 cells failed to recover transcriptional activity over 19 hr. A note of caution: these studies were performed in lymphoblastoid cells with camptothecin, a potent and relatively nonphysiologic stressor that essentially freezes Topo1 on the DNA. But if we can extrapolate from lymphoblastoid cells to neurons, perhaps SCAN1 neurons accumulate SSBs which in turn impede neuronal transcription, thereby compromising neuronal integrity.

These results beg the question: why do neurons, of all the terminally differentiated cells in the body, seem particularly sensitive to SSBs? Due to their extraordinary metabolic demands and long life, neurons may be exposed to sustained, high levels of oxidative stress and, accordingly, suffer increased DNA damage. In light

of this, it is interesting to note that, based on clinical features, brain imaging, and limited neuropathological studies, the most susceptible neurons in AOA1, AOA2, and presumably SCAN1 seem to include large, highly metabolic neurons, such as cerebellar Purkinje cells, motor neurons, and dorsal root ganglia. A second possibility is that neurons are simply more sensitive to accumulated DNA damage than other cell types. It could be, for example, that SSB-induced alterations in transcription are particularly problematic for neurons or that SSBs trigger DNA damage response pathways that in neurons are more prone to promote cell death than recovery (Becker and Bonni, 2004). Neurons also might lack a level of redundancy in SSB repair pathways that exists in other tissues and so are acutely sensitive to its loss in SCAN1. Finally, it is possible that Tdp1 has additional, unique functions in neurons distinct from its DNA repair activities.

A critical next step will be to extend this analysis to actual neurons. Depleting neurons of Tdp1 experimentally will be required for a full understanding of the selective neuronal vulnerability in SCAN1. One can assume that gene targeting strategies, including the generation of knockout mice, are already being pursued. In contrast to mouse knockouts for several other DNA repair enzymes that resulted in embryonic lethality, a *TDP1* gene knockout mouse may be viable given the apparent redundancy for Tdp1 action in replicating tissues. Cre-Lox-mediated conditional knockout approaches, so elegantly employed in a recent study of nibrin function in developing brain (Frappart et al., 2005), would permit a systematic analysis of Tdp1 function in specific brain regions and neuronal subtypes. RNA interference (RNAi) technology can also be harnessed to reduce Tdp1 levels in cultured neurons and in vivo. Viral-mediated RNAi knockdown in the cerebellum versus other brain regions could address regional susceptibility.

If Tdp1-deficient neurons are shown to accumulate SSBs, it will be critically important to determine whether this genotoxic stress induces a DNA damage response or alters the neuronal transcriptome, as one might predict from these results. Another important line of inquiry will be to determine whether the AOA1 disease protein aprataxin, which is already linked to SSB repair (Clements et al., 2004; Gueven et al., 2004; Mosesso et al., 2005), ties into Tdp1-dependent processes. While the connection of the AOA2 disease protein senataxin to DNA repair is less well established, its predicted involvement both in RNA processing and in transcription-coupled repair (Ursic et al., 2004) further suggests intriguing links between DNA repair and transcription in neurodegeneration.

Do these results have implications for inherited ataxias *not* directly due to defects in DNA repair? Possibly, given that DNA is a key target of reactive oxygen species, with SSBs being a major form of oxidative damage. For FA and other ataxias in which oxidative stress is considered to be an important pathogenic element, damage to DNA (including but not limited to SSBs) and subsequent transcriptional alterations could prove to be an important downstream consequence of oxidative stress. Remarkably, FA shares some of the same neuropathological hallmarks with DNA repair ataxias, including degeneration of posterior columns and dorsal root ganglia. Unfortunately, relatively little is known about the extent of oxidative DNA damage and transcriptional effects in affected neurons of FA patients and mouse models.

Finally, perhaps these results may even tell us something about pathogenic mechanisms in the age-related dominant ataxias caused by polyglutamine expansion. Fundamentally, these disorders are proteinopathies: that is, the disease protein adopts an abnormal conformation with deleterious consequences for the neuron (Ross and Poirier, 2004). In light of the previous discussion, however, three general observations about polyQ diseases seem pertinent to the potential role of DNA damage in this class of diseases: many polyQ disease proteins shift from the cytoplasm to the nucleus during disease; transcriptional dysregulation (most often repression) commonly occurs; and there is some, albeit limited, evidence for oxidative DNA damage (Bogdanov et al., 2001). Conventional wisdom has it that transcriptional failures in polyQ disease reflect aberrant interactions of polyQ proteins with nuclear transcriptional machinery (Schaffar et al., 2004, and references therein). Perhaps it is now time to look more carefully at DNA damage and its downstream consequences as a potential contributor to pathogenesis in these and other neurodegenerative diseases.

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