Senataxin protects the genome
Implications for neurodegeneration and other abnormalities

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Ataxia oculomotor apraxia type 2 (AOA2) is a rare autosomal recessive disorder characterized by cerebellar atrophy, peripheral neuropathy, loss of Purkinje cells and elevated α-fetoprotein. AOA2 is caused by mutations in the SETX gene that codes for the high molecular weight protein senataxin. Mutations in this gene also cause dominant neurodegenerative disorders. Similar to that observed for other autosomal recessive ataxias, this protein protects the integrity of the genome against oxidative and other forms of DNA damage to reduce the risk of neurodegeneration. Senataxin functions in transcription termination and RNA splicing and it has been shown to resolve RNA/DNA hybrids (R-loops) that arise at transcription pause sites or when transcription is blocked. Recent data suggest that this protein functions at the interface between transcription and DNA replication to minimise the risk of collision and maintain genome stability. Our recent data using SETX gene-disrupted mice revealed that male mice were defective in spermatogenesis and were infertile. DNA double strand-breaks persisted throughout meiosis and crossing-over failed in SETX mutant mice. These changes can be explained by the accumulation of R-loops, which interfere with Holiday junctions and crossing-over. We also showed that senataxin was localized to the XY body in pachytene cells and was involved in transcriptional silencing of these chromosomes. While the defect in meiotic recombination was striking in these animals, there was no evidence of neurodegeneration as observed in AOA2 patients. We discuss here potentially different roles for senataxin in proliferating and post-mitotic cells.

Autosomal recessive cerebellar ataxias are a class of progressive neurodegenerative disorders that result from cerebellar atrophy and spinal tract dysfunction.1 One of these, ataxia oculomotor apraxia type 2 (AOA2) is characterized by progressive cerebellar atrophy and peripheral neuropathy, oculomotor apraxia and elevated α-fetoprotein serum levels, with an onset between 10–20 y of age.1-4 Brain MRI reveals diffuse cerebellar atrophy and electrophysiology confirms the peripheral neuropathy.4 The major clinical features of this disorder are shown in Table 1. In a post-mortem AOA2 case, Criscuolo et al.5 observed reduced brain size and cerebellar atrophy which was most evident at the level of the vermis and anterior lobe; the cerebellar cortex had marked loss of Purkinje cells and brainstem and spinal cord were slightly reduced. Thus, as with other autosomal recessive ataxias, pathology in the cerebellum features strongly. However, unlike that for the related disorder ataxia telangiectasia (A-T), there is no evidence of increased cancer susceptibility in AOA2.

The gene mutated in AOA2 was initially mapped to chromosome 9q34 and subsequently identified as SETX. SETX is predicted to code for a 2,667 amino acid protein (senataxin) that contains a highly conserved C-terminal seven-motif domain found in the superfamily 1 of...
DNA/RNA helicases and an N-terminal domain important for protein-protein interaction.24 Generally speaking, mutations in a single gene, such as $SETX$, gives rise to one syndrome, which, of course, may show heterogeneity depending on the nature and localization of the mutations. In the case of $SETX$, up to 4 different syndromes are associated with mutations in this gene (Table 2). Juvenile amyotrophic lateral sclerosis (ALS4) is a form of juvenile ALS characterized by distal muscle weakness and atrophy, normal sensation and pyramidal tract signs. The ALS4 locus maps to chromosome 9q34. Chen et al.9 detected missense mutations in this gene ($SETX$), which possesses helicase activity, and is involved in the processing of tRNA, rRNA, small nuclear and small nucleolar RNA.12 Sen1p also interacts with Rad2, which is required for DNA repair, suggesting that the protein may be involved in protecting the genome.13 We demonstrated that this might also be the case for senataxin by showing that AOA2 patient cells display sensitivity to DNA damaging agents such as $H_2O_2$, camptothecin and mitomycin C and the cells had elevated levels of oxidative DNA damage.8 In support of a role for senataxin in the DNA damage response, it has also been demonstrated that telomere length is constitutively reduced in AOA2 lymphocytes and the rate of telomere shortening by DNA damage is increased in these cells.14 Interaction of Sen1p with Rnt1p (an endoribonuclease required for RNA maturation) suggested that Sen1p is also involved in RNA processing and transcription.15 We provided evidence for a similar role in human cells by identifying novel senataxin-interacting proteins, the majority of which are involved in transcription and RNA processing, including RNA polymerase II.15 Binding of RNA polymerase II to candidate genes was significantly reduced in senataxin deficient cells and this was accompanied by decreased transcription of these genes, suggesting a role for senataxin in the regulation/modulation of transcription. RNA polymerase II-dependent transcription termination was defective in cells depleted of senataxin in keeping with the observed interaction of senataxin with poly(A) binding proteins 1 and 2. Splicing

### Table 1. Ataxia Oculomotor Apraxia Type 2 (AOA2): Clinical Features

| Onset second decade |
| Diffuse cerebellar atrophy (MRI) |
| Peripheral neuropathy |
| Early loss of reflexes |
| Loss of Purkinje cells |
| Oculomotor apraxia |
| Extra-neurological features of A-T missing but α-fetoprotein elevated |
| Autosomal recessive cerebellar ataxia |

### Table 2. Mutations in $SETX$ give rise to different neurodegenerative disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Age of onset (yr)</th>
<th>Major Clinical Phenotype</th>
<th>Gene/Protein</th>
<th>Inheritance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia oculomotor apraxia type 2 (AOA2)</td>
<td>10–20</td>
<td>Cerebellar ataxia with peripheral neuropathy</td>
<td>Setx/senataxin</td>
<td>Recessive</td>
<td>1–5</td>
</tr>
<tr>
<td>Tremor ataxia syndrome (TAS)</td>
<td>3, 13*</td>
<td>Cerebellar ataxia without peripheral neuropathy</td>
<td>Setx/senataxin</td>
<td>Dominant</td>
<td>11</td>
</tr>
<tr>
<td>Juvenile Amyotrophic lateral sclerosis (ALS4)</td>
<td>14**</td>
<td>Limb weakness and severe muscle wasting</td>
<td>Setx/senataxin</td>
<td>Dominant</td>
<td>9</td>
</tr>
<tr>
<td>Autosomal dominant proximal spinal muscular atrophy (ADSMa)</td>
<td>10–20</td>
<td>Muscular atrophy and weakness</td>
<td>Setx/senataxin</td>
<td>Dominant</td>
<td>10</td>
</tr>
</tbody>
</table>

*Age of onset for daughter and mother. **Average age of onset.
efficiency of specific mRNAs and alternate splice-site selection of both endogenous genes and artificial minigenes were altered in senataxin-depleted cells. A role for senataxin in transcription elongation and termination is further supported by a report showing that cells with senataxin knockdown displayed an increase in RNA read-through and Pol II density downstream of the Poly (A) site and also exhibited increased levels of R-loop formation. R-loops are RNA/DNA hybrids that form over transcription pause sites by interaction with a ssDNA template behind an elongating Pol II complex (Fig. 1). These structures are potentially harmful and can cause genomic instability if left unresolved. The yeast ortholog of senataxin, Sen1p, has also been shown to protect its heavily transcribed genome from R-loop-mediated DNA damage. More recently, Hazelbaker et al. showed that kinetic competition between elongating RNA pol II and Sen1p helicase likely explains the temporal and spatial window for early Pol II termination. Loss of Sen1p results in transient R-loop accumulation, giving rise to transcription-associated recombination and genome instability. More insight into this was provided recently by Alzu et al. who showed that Sen1p associates with DNA replication forks to protect their integrity across RNA Pol II–transcribed genes. Thus, Sen1p plays an important role in coordinating replication with transcription to protect the genome. Support for a similar role for the human ortholog, senataxin, was provided recently by Yuce-Petronczki and West who showed that senataxin localized to distinct nuclear foci in S/G2 phase cells and that the number of these foci increased in response to impaired replication. These data suggest that senataxin localizes to collision sites between the transcription apparatus and components of the replisome.

All of the investigations above have described a role for senataxin in preventing collision between DNA replication forks and ongoing transcription to preserve genome integrity in proliferating cells. However, the major phenotype in AOA2 patients is progressive neurodegeneration in post-mitotic tissue. Under those conditions, ongoing transcription will not encounter DNA replication forks. What then is the role of senataxin in the brain? Vantaggiato et al. provided evidence that senataxin plays a role in neuritogenesis and cytoprotection during neuronal differentiation which is mediated by fibroblast growth factor 8. However, since this is a progressive disease, it is unlikely that the role of senataxin is restricted to development. To address this further, we disrupted the SETX gene in a mouse model for AOA2, which was the subject matter of our recent publication. Unfortunately, we did not observe a neurodegenerative phenotype in the Setx−/− mice and there was no evidence of more subtle behavioral differences in these mice, which limited investigation into the nature of the defect in the brains of these mice. This was not altogether surprising since knock-out of several of the genes causing autosomal recessive ataxias in humans fails to re-capitulate the phenotype in mouse

Figure 2. Accumulation of R-loops in Setx−/− germ cells. (A) Immunostaining of testes cross-sections from wildtype (+/+) and Setx knockout (−/−) mice with the S9.6 DNA/RNA (R-loop) antibody. Nuclei were stained with Hoechst 33342. Scale bar, 100 μm. Regions 1 and 2 show magnifications. (B) Immunostaining of pachytene spermatocytes from wildtype (+/+) and Setx knockout (−/−) with S9.6 antibody shows a dramatic accumulation of R-loops in senataxin deficient germ cells. SCP3 was used to stain for the synaptonemal complex and identify pachytene stage cells. Scale bar, 20 μm.
subsequently showed that senataxin had an essential role in spermatogenesis in mice and in its absence these cells failed to progress past the pachytene stage of prophase 1 of meiosis. The DNA double-strand breaks (DSB) introduced by Spo11 in readiness for meiotic recombination were inefficiently repaired on autosomes, resulting in a failure to complete crossing-over. During the process of crossing-over, autosomes remain transcriptionally active, so it was possible that in the absence of senataxin, R-loops would accumulate in the vicinity of unrepaired DNA DSB leading to collapse of Holiday junctions and inhibition of the crossing-over step. Indeed this was the case since we detected elevated levels of R-loops in both spermatocyte spreads and testes sections (Fig. 2). Wild-type mice showed a very much reduced level of signal. So in the case of spermatocyte differentiation, the R-loops that accumulate in the absence of senataxin appear to collide with Holiday junctions rather than with advancing replication forks. We also screened for the presence of R-loops in the brains of Setx mutant mice but failed to detect these structures by immunofluorescence (unpublished data). This was not altogether surprising since neither DNA replication nor DNA recombination is taking place in this tissue. It is possible that persistence of DNA damage in post-mitotic cells might lead to the accumulation of these structures, which in turn could contribute to the neurodegenerative changes in AOA2 patients.

However, we and others have provided evidence for a broader role for senataxin in transcription and other cellular processes. Senataxin plays an important role in transcription termination to prevent RNA readthrough, which may or may not be related to R-loop resolution. The presence of significant readthrough of mRNA may lead to inefficient or aberrant protein synthesis and consequently cell toxicity. Senataxin has also been shown to play a role in the regulation of splicing and deficiency of the SR splicing factor ASF/SF2 leads to R-loop accumulation and genome instability. This in turn may interfere with the fidelity of the transcriptome in AOA2 cerebellum.

AOA2 is just one of several neurodegenerative disorders characterized by...

Figure 3. Protection of the genome by senataxin in proliferating vs. post-mitotic cells. (A) In proliferating cells, collision of the transcription apparatus (RNA Pol) with replication forks (DNA Pol), stalled replication forks following DNA damage exposure, or Holiday junctions during homologous recombination, lead to the formation and accumulation of R-loop structures. In the presence of senataxin, R-loops are effectively resolved by its putative DNA/RNA helicase activity, thus leading to normal cellular metabolism and cell survival. In the absence of senataxin, R-loops accumulate and impact on RNA metabolism through the alteration of mRNA splicing, the inhibition of transcription termination, the promotion of readthrough, the alteration of gene expression and the formation of DNA breaks. The accumulation of these defects drives genomic instability and ultimately cell death. (B) In contrast, in post-mitotic cells, such as neurons, the absence of DNA replication and homologous recombination, and the lack of R-loops accumulation suggest senataxin’s role in protecting the genome may be directly due to its effect on mRNA splicing, transcription termination and the modulation of gene expression through its interaction with RNA binding proteins.

models. However, we observed that Setx−/− male mice were infertile and fertility was reduced in females. While there is no information on male fertility in AOA2, there are a few reports of hypogonadism in females. Criscuolo et al. reported two patients who entered menopause in early adulthood which was also observed in a separate study. Ovarian failure has also been observed in a patient with AOA2 and another patient had a diagnosis of polycystic ovarian syndrome. We...
defects in RNA metabolism that impact either gene transcription, pre-mRNA splicing, ribonucleoprotein complex formation, mRNA transport, RNA translation or RNA degradation. One form of the motor neuron disease, amyotrophic lateral sclerosis (ALS) is caused by defects in TDP-43 and FUS/TLS, both of which contain RNA-binding motifs and spinal muscular atrophy (SMA) is caused by deletion or mutation in survival of motor neuron 1 (SMN1). Profound loss of splicing integrity is a critical mechanism common to ALS and SMA. It is also of interest that we previously identified SMN as one of the proteins that interact with senataxin, pointing to an overlapping role in RNA processing. In summary, much progress has been made recently on the function of senataxin and its yeast ortholog Sen1p in resolving potential conflict between transcription and DNA replication in proliferating cells. Resolution of R-loops is prominent in that role but it is evident that senataxin has a broader involvement in RNA processing (Fig. 3). Its role in post-mitotic cells is not clear, but it is likely that this will be on some aspect of RNA metabolism to protect the integrity of the genome/transcriptome. What remains intriguing is how mutations in a single gene SETX, often in close proximity to one another, can cause both autosomal dominant and recessive disorders. However, while mutations in SETX give rise to what appears to be 4 different syndromes, it is also evident that there is some overlap across these disorders. To date, several senataxin-interacting proteins have been identified, all of which are involved in some aspect of RNA processing. Mutations in some of these also give rise to other neurodegenerative disorders. It seems likely that these proteins function in complexes to control RNA metabolism and different mutations in the various subunits may impact differently on the function of the complex(es), giving rise to the heterogeneity of neurodegenerative disorders observed. The challenge that lies ahead is to understand the relationships of these proteins to one another in the complexes and how they function to control processes in neurons and other cell types in the brain to minimise the risk of neurodegeneration.

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