

# Human Senataxin Dysfunction in Neurological Disorders and R-loops Resolution: an Overview.

Lucas Mesquita Cardoso <sup>a</sup>

<sup>a</sup> Faculty of Biomedical Science, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil.

**Abstract.** Human senataxin (SETX) is a Helicase DNA-RNA protein located in the cell nucleus, which regulates transcription, promotes the solution of R-loops and stress granules, as well as acts in the neuroprotection. Missense mutations in the SETX gene can result in both loss and gain of function, activating cell death pathways, leading to neurodegeneration associated with Ataxia with Oculomotor Apraxia type 2 (AOA2) or Amyotrophic Lateral Sclerosis type 4 (ALS4). ALS4 is an autosomal dominant neuropathy characterized by the selective loss of motor neurons in the spinal cord, brainstem, and cerebral cortex, leading to hyperreflexia and muscle atrophy. AOA2 has a recessive inheritance and is manifested by cerebellar ataxia, cerebellar atrophy, axonal sensorimotor neuropathy, and oculomotor apraxia. The pathogenesis of the two neuropathies are still not elucidated, but could be associated with an impairment in its R-loops resolution role caused by mutations.

**Keywords.** Senataxin, Ataxia with Oculomotor Apraxia, Amyotrophic Lateral Sclerosis, R-loop.

## 1. Introduction

Human senataxin (SETX) is a protein with 2677 amino acids and two domains evolutionarily preserved in chordate organisms. One of them is described as a DNA-RNA helicase domain (residues 1,931-2,456) and the other as an N-terminal region (residues 1-668), homologous to the Sen1p2 protein from *Schizosaccharomyces pombe* [1, 2]. SETX is found mainly in the cell nucleus [3].

The role of SETX in cellular functioning is associated with the regulation of transcription, the reduction of R-loops and neuroprotection [4]. Its importance is also described, relating it to the preservation of genomic integrity during spermatogenesis [5] and to the delay in the aging of female germline cells [6].

After extensive SUMOylation by SUMO 2/3 in the N-terminal region, SETX contributes to curated RNA manipulation together with exosome component 9 (Exos9), promoting transcription quality control. In the S-phase of the cell cycle, SETX forms nuclear foci at points between the replisome and the RNA polymerase II (RNAP II) machinery, reducing replication stress. RNA helicases, including SETX, promote the dissolution of stress containers, composed of proteins and high concentrations of untranslated mRNA. Therefore, the adaptive response to oxidative stress can be influenced by dysfunctions in the performance of SETX [7].

Neuropathies can be triggered by missense mutations in the SETX gene, specifically amyotrophic lateral sclerosis type 4 (ALS4) and ataxia with oculomotor apraxia type 2 (AOA2) [2, 8]. The loss of function of SETX caused by mutations promotes the pathogenesis of AOA2, while the gain of function is responsible for ALS4 [2, 9–11].

## 2. Ataxia with Apraxia Oculomotor type 2

AOA2 is characterized by the early onset of cerebellar ataxia and axonal sensorimotor peripheral neuropathy [12]. Other clinical aspects include dysarthria (whose presence can be identified in 100% of individuals with AOA2), nystagmus (91%) and strabismus (30%) [13], as well as tremors (14%), dystonic postures (13.5%) and chorea (9.5%) [14]. Serum  $\alpha$ -fetoprotein is elevated in more than 90% of affected individuals. Other possible biochemical changes include high levels of serum cholesterol and creatine kinase [15].

According to a study carried out by Anheim and collaborators [14], AOA2 develops on average at 14 years of age. Its prevalence in the Alsace region, in France, was estimated at 1 case for every 900,000 inhabitants, making it the second most common cerebellar ataxia [16].

Oculomotor apraxia is an inconstant symptom,

present in approximately 50% of patients with AOA2 [12], with the name suggested by Duquette and his collaborators [13]: spinocerebellar ataxia, autosomal recessive, with axonal neuropathy type 2 (SCAN2).

Despite the continued expansion of the number of genes associated with cerebellar ataxias [17–20], little is known regarding the molecular basis of its pathogenesis, as observed with AOA2. Knowledge of pathogenesis could support the development of new therapies. Studies at the molecular level provide ways to identify the mechanisms affected by mutations for further investigation [21].

According to Anheim and colleagues [14], in patients with AOA2, pyramidal signs and dystonia are more frequent and the disease is less severe with missense mutations in the helicase domain of the senataxin gene than with missense mutations outside the helicase domain, deletion or nonsense mutations. Thus, they suggested that the lack of pyramidal signs in most patients may be the result of severe motor neuropathy.

### 3. Amyotrophic Lateral Sclerosis type 4

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease characterized by the selective loss of motor neurons in the spinal cord, brain stem and cerebral cortex, leading to muscular atrophy and death from respiratory failure during the degenerative process within 2 to 5 years of diagnosis [22]. Familial ALS accounts for approximately 5% to 10% of ALS cases [23]. The mechanism of toxicity to the motor neuron remains unknown [24].

However, it is known that ALS4 has dominant inheritance [25] and typically develops before the age of 25 [8]. It is characterized by the early onset of weakness of the distal muscles, difficulty walking, hyperreflexia and muscle atrophy [26].

### 4. R-Loops

During DNA replication or gene transcription, DNA undergoes a series of changes that can generate structural intermediates that can facilitate mutations in DNA. An example of such structural intermediates are R-loops, hybrids of RNA and DNA generated during transcription when the non-coding DNA strand is displaced as the transcribed DNA strand forms a hybrid with the nascent pre-mRNA [27, 28]. The formation of R-loops can impact several biological processes, such as antibody diversification in B cells, DNA damage response, and gene expression at multiple levels, including regulation of chromatin architecture in the promoter region, transcription elongation, and termination [29–31].

In human cells, genes that contain CpG islands in promoters are characterized by having R-loops,

which form from the transcription start site to the first exon-intron junction [32–34]. R-loop formation in these gene promoters prevents DNA methylation and promotes a permissive status for chromatin transcription, which ultimately leads to gene expression [35]. An additional site of genomic relevance for the formation of R-loops is represented by gene transcription terminators. In these genomic elements, the formation of R loops induces RNA polymerase II to pause, facilitating efficient transcription termination [36]. Furthermore, the formation of R-loops in terminators triggers the recruitment of the enzyme responsible for conferring the repressive mark H3K9me2, an epigenetic characteristic of the terminator elements of certain highly expressed genes [37].

Although physiologically relevant for many biological processes, the persistence of R-loops can be detrimental to cell viability [38–40]. Generally, exon-intron sequences act as a barrier to R-loop propagation in genomes and splicing factors, such as SLU7 and SRSF1, which prevent R-loop formation at splice sites, allowing proper RNA processing [41–43]. R-loop processing is catalyzed by two main classes of enzymes: RNA-DNA helicase enzymes and nucleases. RNA-DNA helicase enzymes (BLOOM, DDX5, DDX19, DDX21, SETX, WERNER, among others) catalyze the unwinding of the RNA-DNA hybrid part of the R-loop, promoting its resolution, while some RNA-DNA nucleases, such as RNaseH1 and RNaseH2, hydrolyze the RNA portion in RNA-DNA hybrids, thus dissolving R-loops. Other endonucleases involved in R-loop resolution are FEN1, which is capable of cutting both the displaced ssDNA and the RNA strand of an R-loop, and XRN2, which degrades nascent RNA from the 3' terminal cleavage site [2, 44].

### 5. Conclusion

Studies show the importance of R-loops resolution to cell viability and the SETX's role in solving R-loops. Nevertheless, the comprehension of how SETX dysfunction could be the cause of neurological disorders, such as AOA2 and ALS4 is not well understood. Therefore, this theme needs more studies to elucidate the pathogenesis of those two diseases.

### 6. References

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