Alteration of paraspeckles in ALS muscle cells

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by motor neuron degeneration and progressive skeletal muscle atrophy. 90% of cases are sporadic (sALS) and over 50 causative gene mutations have been identified in familial ALS (fALS) such as mutations of the C9orf72, SOD1, TDP43 and FUS genes; but no curative treatment exists[1].

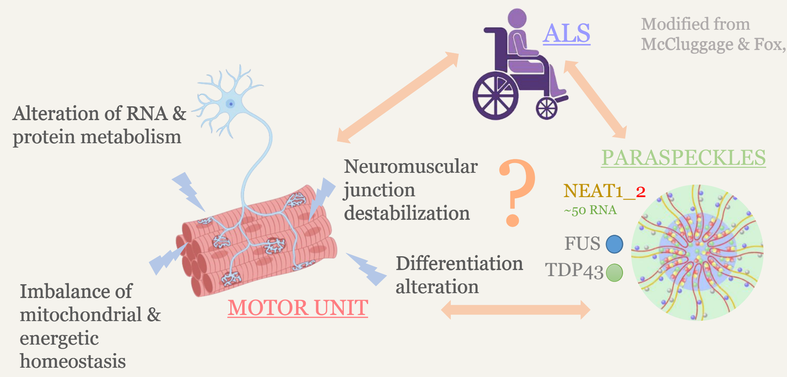
Despite ALS being known as the Motor Neuron Disease, muscles undergo a lot of alterations in several biological processes such as RNA processing, mitochondrial homeostasis and myogenesis[2].

Among the proteins mutated in ALS, several like FUS and TDP43 are involved in the formation of membraneless subnuclear bodies called paraspeckles. Paraspeckles are formed by the long non-coding RNA NEAT1_2 and RNA-binding proteins[3] and function as molecular sponges.

Several links have been established between paraspeckles and ALS :

- The RNA or proteins from the most frequently mutated genes in ALS (C9orf72, SOD1, TDP43, FUS) are associated to paraspeckles.
- Paraspeckles regulate many pathways altered in ALS including mitochondrial homeostasis and myogenesis[4]
- Paraspeckles are upregulated in ALS motor neurons[4].

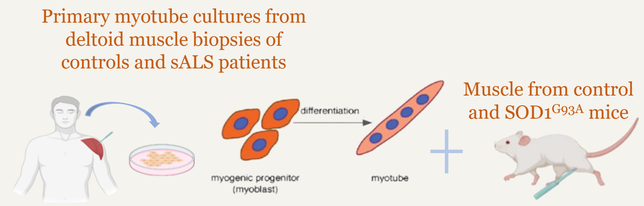
However, their status in the ALS muscle is unknown. Therefore we ask ourselves whether paraspeckles could be altered in ALS and involved in ALS physiopathology and the degeneration of the motor unit especially at the level of muscle cells.



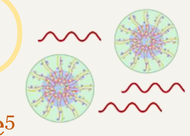
Aim

Characterize the status and role of paraspeckles in ALS muscles

Samples



RNA extraction



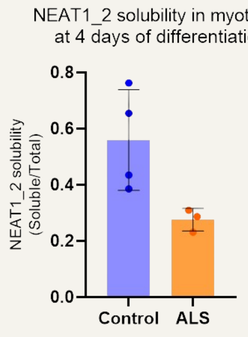
NEAT1_2 is semi-extractible[5]

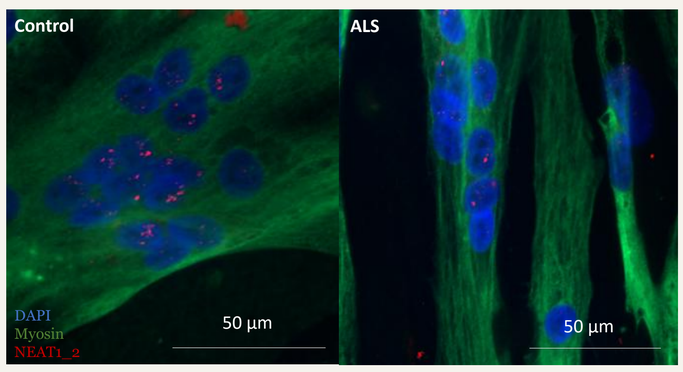
- Classical trizol extraction = soluble NEAT1_2
- Heat trizol extraction = total NEAT1_2

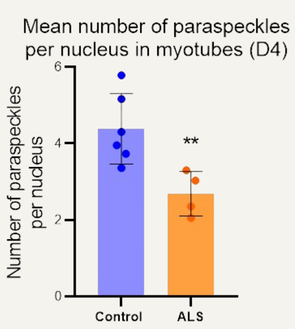
Results

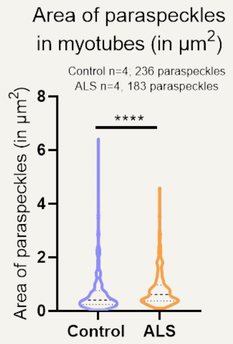
In vitro

Status









In vitro, NEAT1_2 is less soluble in ALS myotubes.

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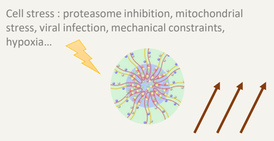
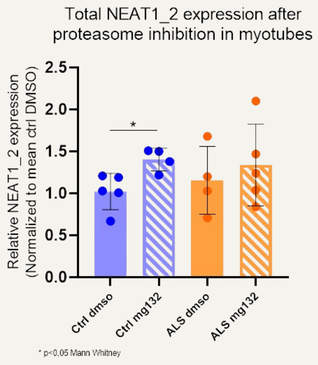
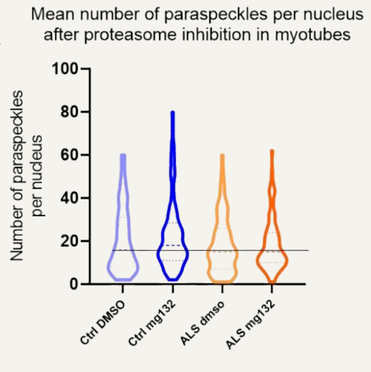
In vitro, ALS myotubes have significantly less paraspeckles than controls and these paraspeckles are significantly bigger.

Function



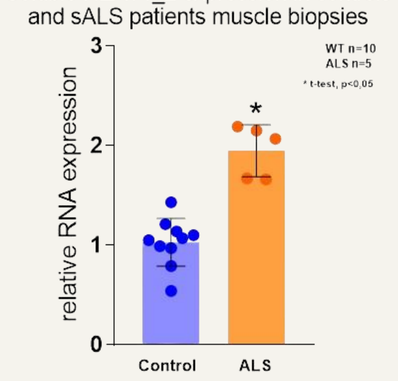
In vitro, NEAT1_2 expression and paraspeckle number increase in controls in response to cellular stress.





BUT Paraspeckles in ALS myotubes do not respond to cell stress.

In vivo



In vivo, soluble NEAT1_2 is significantly overexpressed in muscles of both sALS patients & fALS mice.

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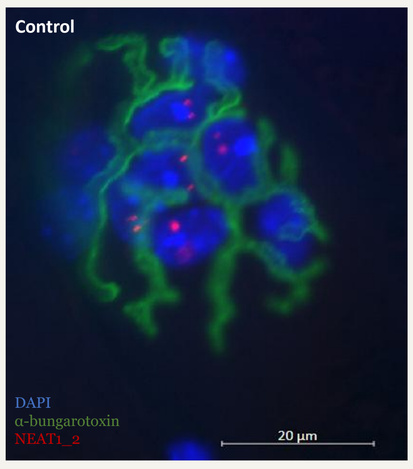
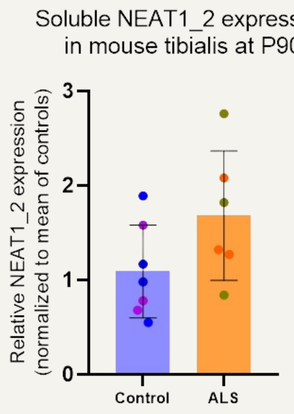
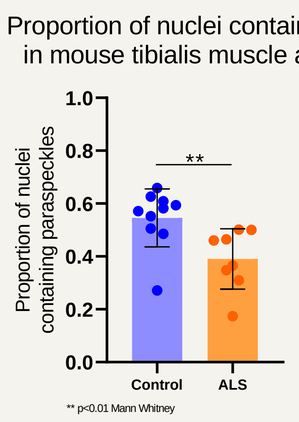
In vivo, soluble NEAT1_2 is overexpressed in ALS mice tibialis at a symptomatic stage (P90).

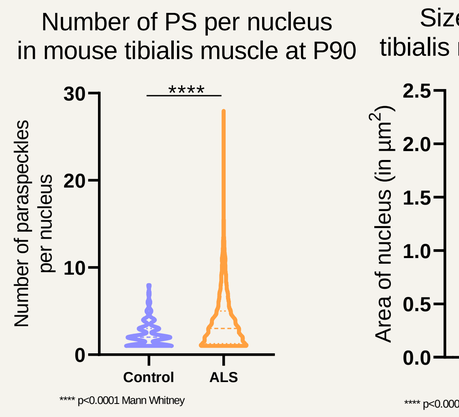
In addition, in ALS mice tibialis at P90, less nuclei contain paraspeckles but nuclei contain more paraspeckles.

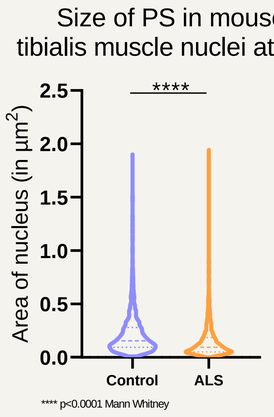
Moreover, paraspeckles are significantly smaller in ALS mice tibialis at P90.











At P90, neuromuscular junctions are degenerated (smaller and more fragmented) in ALS mice.

In addition, in ALS mice tibialis at P90, less nuclei contain paraspeckles but nuclei contain more paraspeckles.

Moreover, paraspeckles are significantly smaller in ALS mice tibialis at P90.

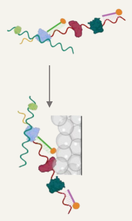
Ongoing

→ Analyze the RNAs that interact with paraspeckles in ALS muscle cells

Capture hybridization Analysis of RNA targets

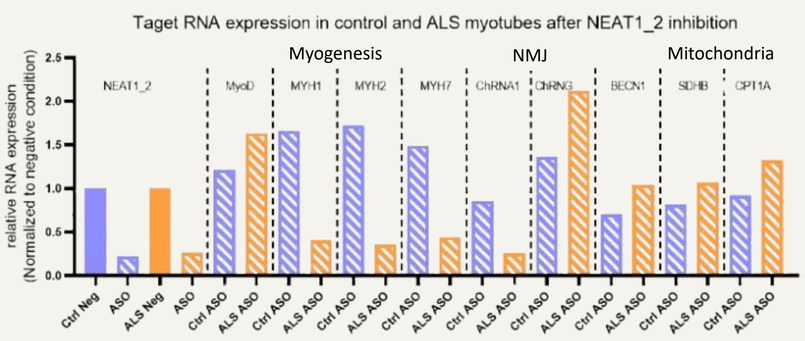
+

RNA RNASeq



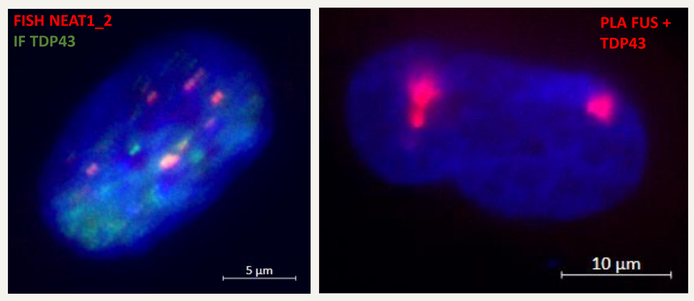
Paraspeckles in control myotubes sequester RNAs involved in :

Myogenesis
Neuromuscular junction
Mitochondrial function
...



These targets are differentialy regulated by paraspeckles in ALS myotubes.

→ Analyze essential protein association to paraspeckles



With IF/FISH colocalisation and proximity ligation assay, we study paraspeckle protein interactions in ALS myotubes.

References

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Conclusion

Altogether, our results show an alteration in paraspeckles in ALS muscles. *In vivo*, we showed an overexpression of soluble NEAT1_2 in ALS patient muscles compared to healthy individuals. In SOD1^{G93A} tibialis, we observed that less nuclei contain paraspeckles but there are more paraspeckles per nucleus and they are significantly smaller independently of NEAT1_2 expression. In human sALS myotubes *in vitro*, we detected a decrease of NEAT1_2 solubility, and a decrease in the number of paraspeckles per nucleus but paraspeckles are significantly bigger than in controls. Moreover, paraspeckles in ALS myotubes do not respond to cellular stress (proteasome inhibition and viral infection) in the same way as in control myotubes. We also identified target RNAs associated to paraspeckles and involved in pathways that are altered in the ALS muscle such as myogenesis or NMJ. These targets' regulation by paraspeckles is altered in ALS myotubes, further showing paraspeckle involvement in cell dysfunctions observed in the disease.

Altogether our results show a dysfunction of paraspeckles that could participate in the development of ALS pathology. We are currently characterizing the status of paraspeckles and their partners both in control and in ALS muscle cells in more details. We are also studying the effects of paraspeckles modulation on different cellular processes that are altered in ALS (myogenesis, mitochondrial homeostasis) to shed light on the role of the membraneless organelles and better understand the physiopathology of ALS. Furthermore we are studying paraspeckle proteins' interactions as they could be key to paraspeckle malformations observed in ALS. Paraspeckles could potentially appear as relevant therapeutic targets or biomarkers of this fatal neurodegenerative disease.