Propagation and toxicity of pathological determinants in Amyotrophic Lateral Sclerosis

7èmes Journées de la Recherche sur la SLA et les Maladies du Neurone Moteur
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INTRODUCTION

Pathological determinants are present in ALS motor neurons and also in glial cells

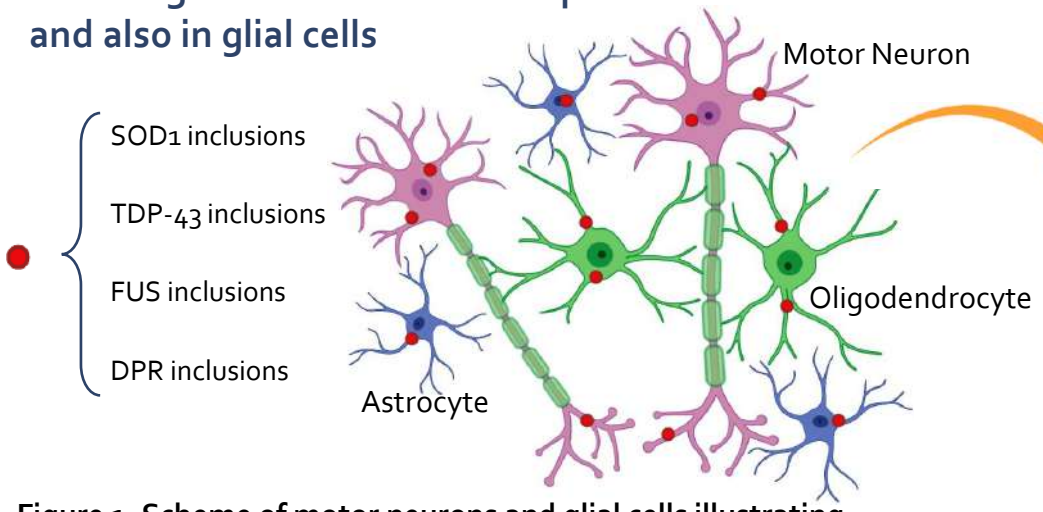


Figure 1. Scheme of motor neurons and glial cells illustrating accumulations of pathological determinants.

ALS onset is anatomically localized and spread to the whole body

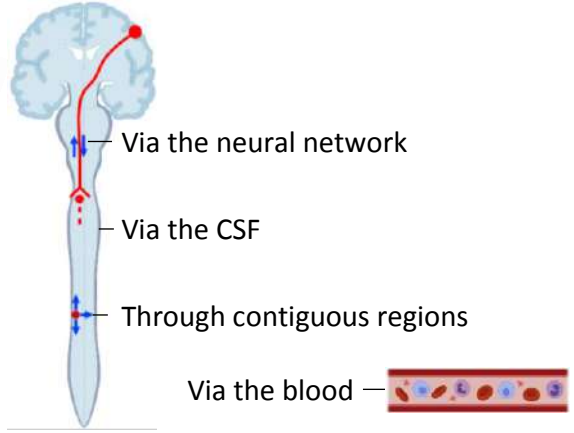
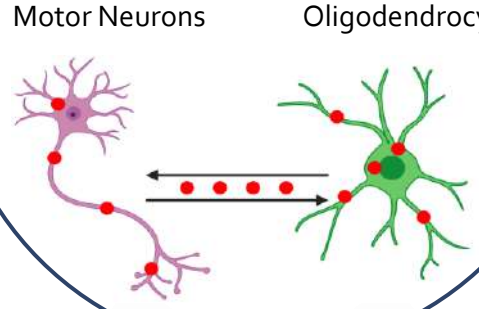


Figure 2. Scheme of the CNS and the different hypothesis of ALS pathology propagation

HYPOTHESIS :

Could the pathological determinants of ALS spread from cell to cell and contribute to the progression of the disease ?



The USP19-dependent MAPS pathway

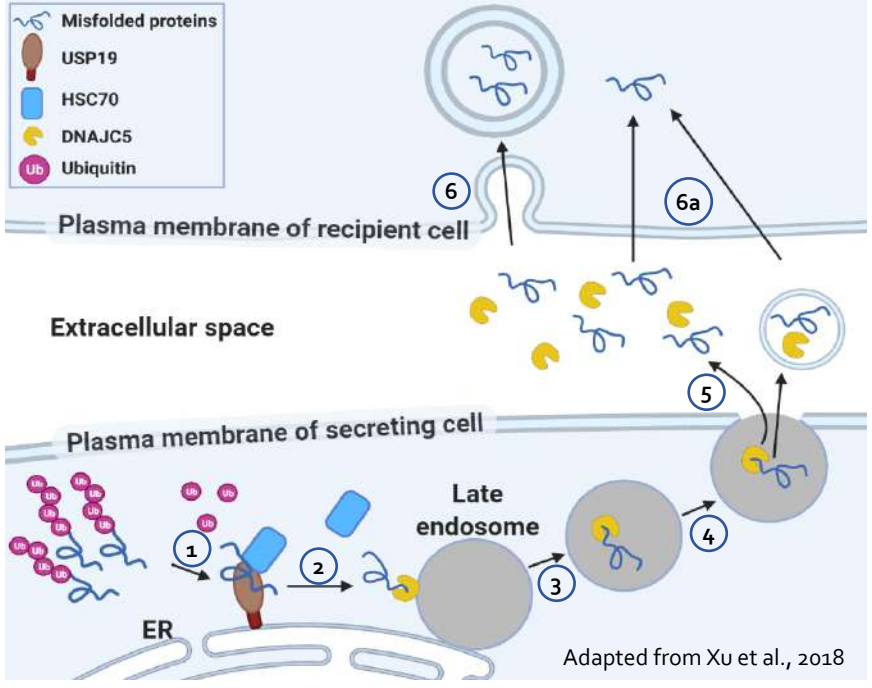


Figure 3. The MAPS pathway

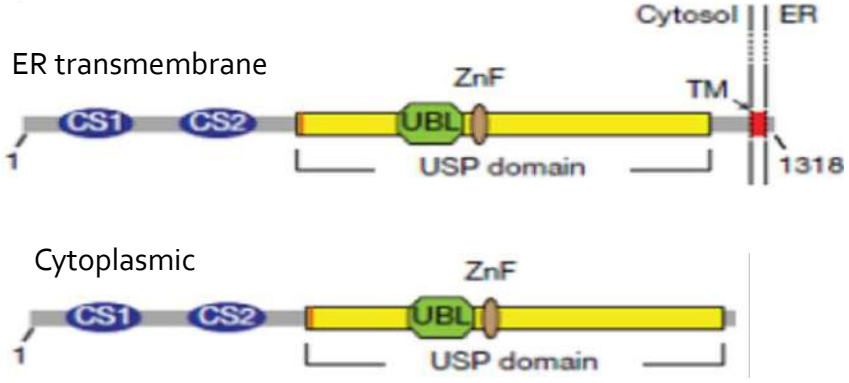
1. A misfolded protein (MP) can be recruited by the deubiquitinase USP19 localized on the ER.

2,3. The MP can then be transferred to the lumen of a late endosome (LE) via a translocation of the DNAJC5-MP complex

4,5. LE moves towards the cell membrane, fusion occurs and the MP can be released in the extracellular space.

6,6a. The MP may enter a recipient cell either by endocytosis or translocation

USP19: Isoforms and roles



Lee et al., 2016

Figure 4. Scheme of USP19 protein.

- Hydrolysis the bonds forms by ubiquitin
- Removing of poly ubiquitin chains from misfolded proteins
- 2 major isoforms: cytoplasmic and ER transmembrane

MODELS

iPSc generation and their derivative neural cells

Age and sex matched healthy **controls**

Isogenic controls

ALS patients mutated in :

- SOD1
- TDP-43
- FUS
- C9ORF72

Somatic cells

Reprogramming

iPSc

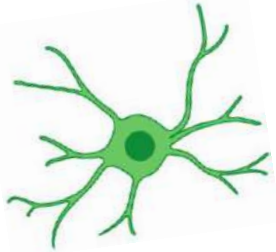
Self renewal

Differentiation

Motor Neurons

Oligodendrocytes Precursor Cells

Maturation

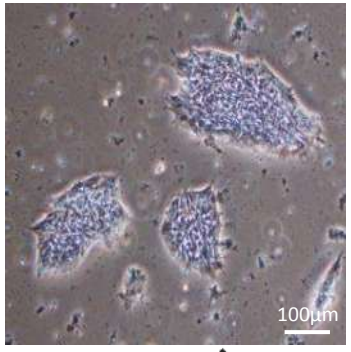


Oligodendrocytes

PROTOCOLS

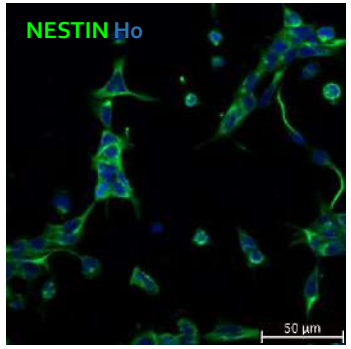
iPSc differentiation into motor neurons and oligodendrocytes

iPSc



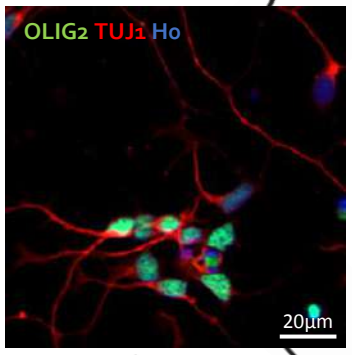
CHIR / LDN
RA / SB / SAG

Neural Stem cell (NPC)



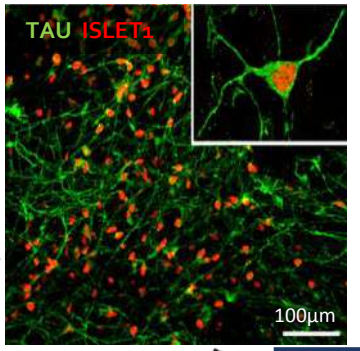
LDN / RA
SB / SAG

Progenitors cells

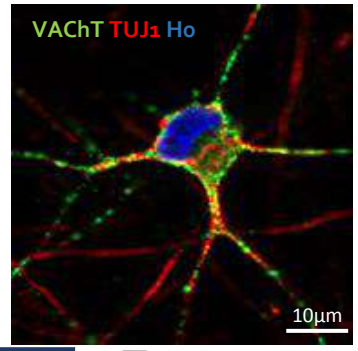


RA / SAG
BDNF/GDNF

Young Motor Neurons



Mature Motor Neurons



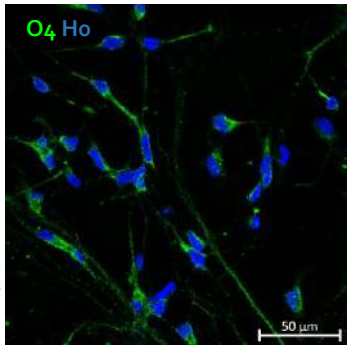
BDNF/GDNF
CNTF / IGF1

MN protocol already set up in the lab

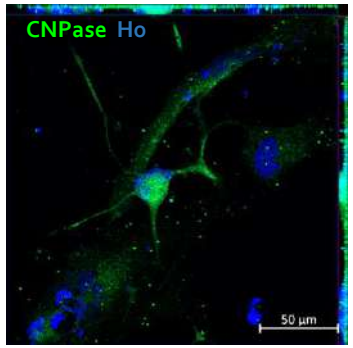
(Maury et al., 2014,
Lefebvre-Omar et al., in preparation)

SAG / FGF2
PDGFRα/RA

OL precursor cell (OPC)



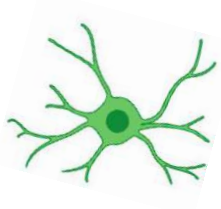
Immature OL



PDGFRα / T3
IGF-1

T3 / IGF-1

Mature OL



Co-cultured with MNs

OL protocol in progress

(Douvaras et al., 2015
Magnani et al., 2019)

RESULTS in MNs

USP19 mRNA and protein expression in progenitors and MNs, and its colocalization with endoplasmic reticulum

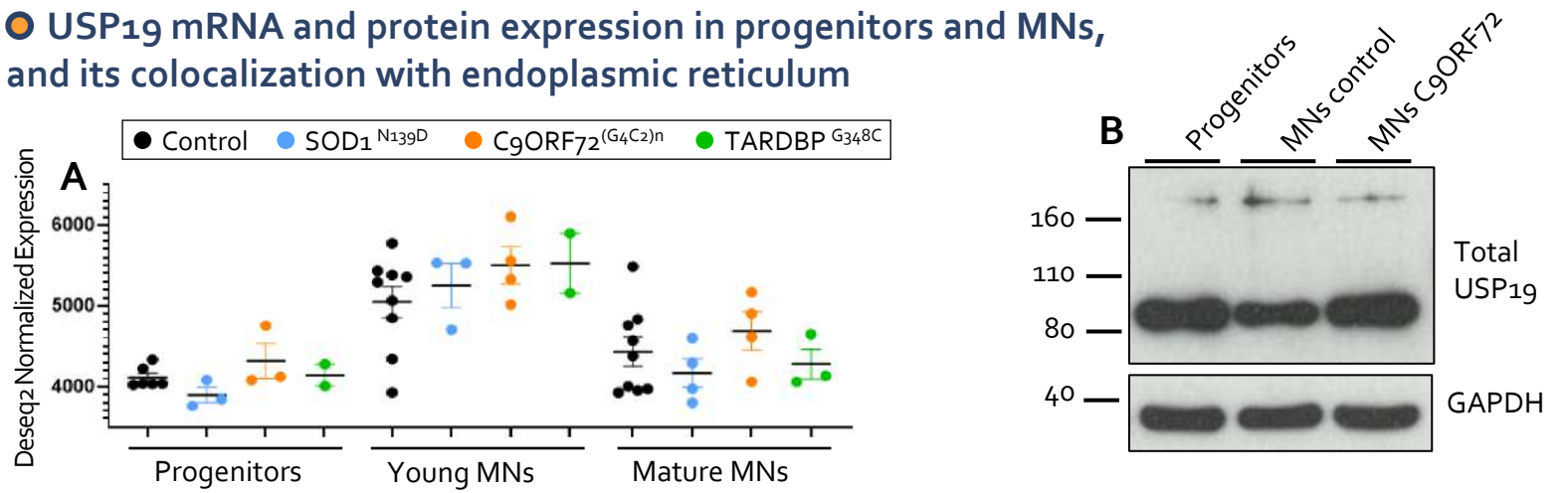


Figure 6. (A) Deseq2 Normalized expression of USP19, **(B)** Western blots showing USP19 protein levels, **(C)** Co-labelling of USP19 and Calnexin, a marker of endoplasmic reticulum.

Expression of ER transmembrane USP19 in MNs

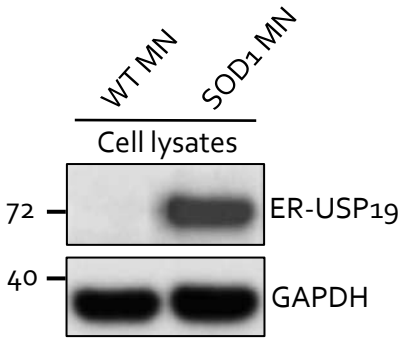


Figure 7. Western Blot showing the expression of transmembrane USP19 only in MNs SOD1, (GAPDH normalizer)

Pathological determinants in MNs

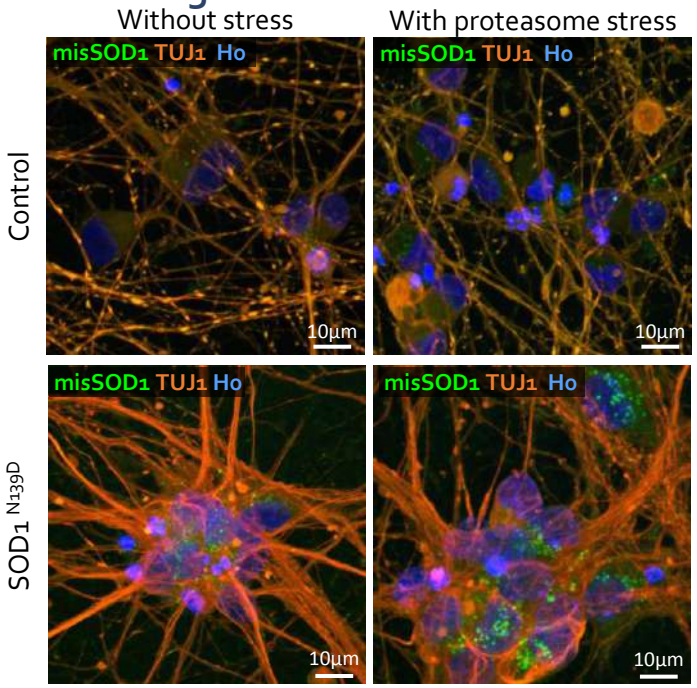


Figure 8. Immunolabelling of misfolded SOD1 in MNs

Secretion of SOD1 in the supernatant of control and SOD1 MNs

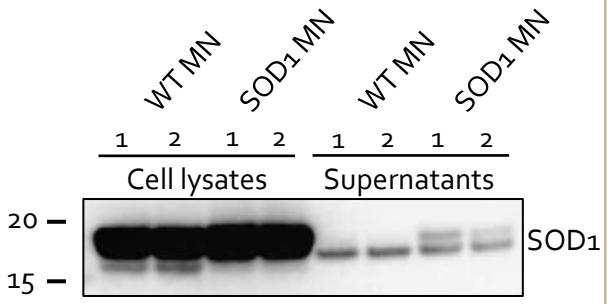


Figure 9. Western Blot of cell lysates and supernatants purified by ultracentrifugation (Théry et al., 2006) of MNs. SOD1 is expressed in cell lysates and is secreted in supernatants.

CONCLUSIONS

- USP19 is expressed in iPSc, progenitors and mature MNs and is expressed near the ER.
- ER transmembrane USP19 is detected exclusively in SOD1 MNs.
- Both control and mutant SOD1 MNs secrete SOD1 in the supernatant.
- A protocol to differentiate iPSc into OPC s and OLs is in progress, coculture will be done with MNs.

PERSPECTIVES

- Search for inclusions (SOD1, TDP43, FUS and DPR) in ALS iPS-derived MNs , OPCs and OLs (normal or stress conditions).
- Analyze the secretion of pathological determinants in MNs, OPCs and OLs.
- Assess the specific USP19 pathway with USP19 siRNA.
- Analyze whether propagation occurs between MNs and OPCs or OLs.