

Amyotrophic lateral sclerosis (ALS) is motor neurodegenerative neuromuscular disease. ALS cases are classified as "sporadic" ALS (sALS) or "familial" ALS (fALS). With the fALS form, the disease is inherited. It has been reported that mutations of genes have been associated with fALS. And for this model, we will focus on the mutated FUS/TLS gene on chromosome 16. The FUS/TLS protein binds to RNA, single-stranded DNA and double-stranded DNA. It also interacts with gene-specific transcription factors and appears in double-strand break sites for repair of DNA damages. Mutations in the FUS NLS (Nuclear localization sequence) impairs the poly (ADP-ribose) polymerase (PARP)-dependent DNA damage response. This impairment leads to FUS aggregate formation which appear to be a common pathologic hallmark for ALS.

AIM: Mutation in the FUS nuclear localization sequence (NLS) induces impairment of poly (ADP-ribose) polymerase (PARP)-dependent DNA damage response and cytoplasmic FUS mislocalization leading to FUS aggregate formation. Along this process, we will develop motor neurons model of FUS-ALS with endogenously tagged proteins.

Methodology

Patient selection

R521C and R521H mutations are the most prevalent mutations within the NLS region of FUS. In addition of normal patients, patients carrying diverse NLS mutations (R521C, R521H) will be selected.

Line cells

Line cells will be established from biopsies (skin or hair cells) obtained after consent from the fALS patients and healthy individuals. The fibroblast lines will be plated in a media, and reprogrammed into induced pluripotent stem cells (hiPSCs) using "Yamanake-factors". These vectors could be transfected into the cells with a transfection agent. These cell lines, then can be constantly regenerated in subcultures and will be monitored until colonies will develop enough.

CRISPR/Cas9 genome editing

To increase the significance of the research, mutations which have been linked to ALS will be performed using CRISPR/Cas9 vector and guide RNAs (gRNA) to create double strand break at the target site and insert the FUS mutated gene with mutation in the C-terminal nuclear localization sequence (NLS) (see figure 1 – GFP-FUS mutants).

Study

Electrophysiology of motor neurons will be performed to check the presence of voltage-gated Na⁺ and K⁺ channels, action potentials and intracellular calcium. After extended maturation of the cultures, we will look for an increase for FUS aggregates and will check for occurrence of DNA double strand breaks in lines of normal cells extracted from healthy patients compared to the mutated lines.

References:

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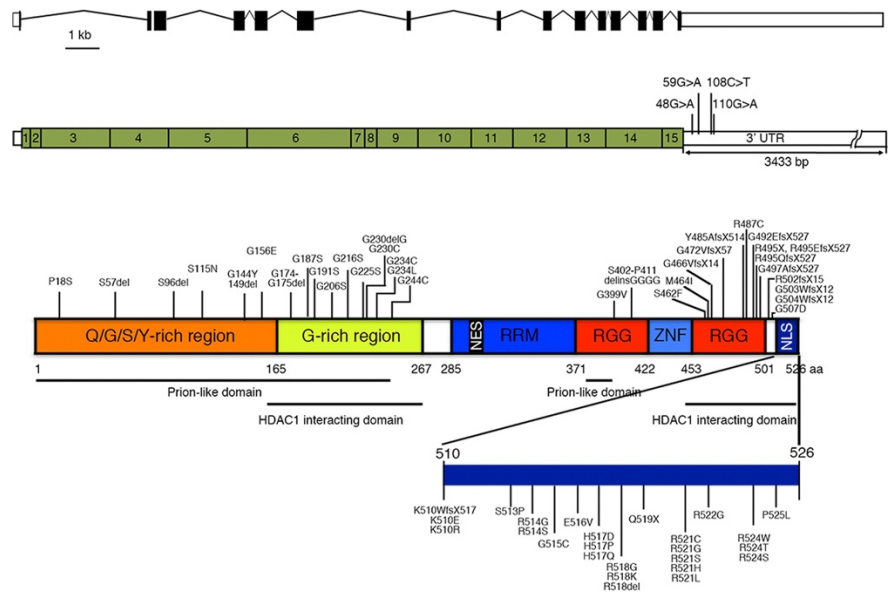


Fig1. FUS NLS mutations

Mutation	Nucleotide change	Exon	Patients	Controls
p.Gly 171_174del	*c.515delGAGGTGGAGGTG	5	1/1009	0/621
p.Gly 173_174del	*c.508delGAGGTG	5	2/1009	3/621
p.G191S	c.571G → A	6	1/1009	0/793
p.R216C	c.646C → T	6	1/1009	0/793
p.Gly 223ins	*c.667insGGC	6	0/1009	2/793
p.Gly 223_224ins	*c.667insGGCGGC	6	0/1009	1/793
p.Gly 223del	*c.679_681delGGC	6	2/1009	0/793
p.Gly 223_Gly224del	*c.679_684delGGCGGC	6	4/1009	0/793
p.G225V	c.674G → T	6	1/1009	0/793
p.G226S	c.676G → A	6	0/1009	1/793
p.Gly 227_Gly230del	c.680_691delGGCGGCGGTG	6	1/1009	0/793
p.Gly 227_Gly229del	c.679_687delGGCGGCGGT	6	1/1009	1/793
p.Gly 228_Gly229del	c.682_687delGGCGGT	6	1/1009	0/793
p.Gly 229_230del	*c.685_690delGGTGGT	6	0/1009	1/793
p.G230C	c.688G → T	6	1/1009	0/793
p.R234C	c.700C → T	6	1/1009	0/793
p.G507D	c.1520G → A	14	2/1009	0/500
p.R521C	c.1561C → T	15	2/1009	0/538

Fig 2 . R521C nucleotide change: C->T