Understanding the function of SIGMAR1 gene in neurons using TALEN-mediated mutagenesis

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What is Amyotrophic Lateral Sclerosis?

- 5th most prevalent neurodegenerative disorder
- Loss of motor neurons
- Leads to muscle weakness, paralysis, and eventually death
- 10% of cases are familial
Symptoms of ALS

- Muscle weakness
- Fasciculation and cramping
- Thick speech
- Shortness of breath
- Difficulty breathing/swallowing
- Death in approximately 3-5 years
Cause of ALS?

• 20% of familial mutations - SOD1

• Sequence genomes to find additional mutations linked to other familial cases
  • Identified SIGMAR1
    • Is this gene causative?
    • If so, what is SIGMAR1 doing in motor neurons?
SIGMAR1

- Sigma non-opioid intracellular receptor 1
- Vesicle trafficking
- Regulates ion channels
- Ca2+ signaling
- Role in motor neuron function and disease?
Animal Model: Zebrafish

• Why use zebrafish?
  • Embryo is easily accessible
  • Big enough for injections
  • Mature quickly
TALENs

- Transcription activator-like effector nucleases
- TAL effectors found in plant pathogenic bacteria
- Functionalized proteins
- Targets specific sequence in gene of interest
- Induces double stranded breaks
- Repair leads to mutations
What are TALENs?
Construction of SIGMAR1 TALEN

SIGMAR1 gene structure

Start
ATG

TALEN targeted

1 2 3 4
SIGMAR1 Mutation in Fish

- Made RNA from DNA
- SIGMAR1 TALEN injected at 25 ng/ul
- Results showed the fish did not have mutations

1: SIGMAR1 control
2: SIGMAR1
Where is SIGMAR1 expressed during zebrafish development?

In Situ Hybridization
Summary

- ALS is characterized by the loss of motor neurons in the brain and spinal cord.
- Mutations in SIGMAR1 receptors have been found in ALS patients.
- TALENs have the potential to mutate genes like SIGMAR1.
Future Directions

• Create SIGMAR1 CRISPR
• Mate mutant fish with MNX1 GFP zebrafish
• Evaluate motor neuron growth and function
  • Swimming test
• Use ALS-specific mutation fish for drug screening

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