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Amyotrophic Lateral Sclerosis (ALS)
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- Degeneration of both upper and lower motor neurons
- Initiates in midlife
- Progresses to paralysis and death
- Genetic inheritance 10% (familial ALS)

Diagram: 90% sALS, 10% fALS
Hallmarks of both forms

• Progressive muscle weakness

• Atrophy and spasticity

• Degeneration and death of upper and lower motor neurons in the brain and spinal cord

• Denervation of the respiratory muscles and diaphragm
Genetics of ALS

- SOD1 is the major gene in ALS comprising 2% of all cases
- All the other known genes account for only 0.1% (*ALS-like diseases*)
  - **ALS2** *(Alsin)*
  - SETX
  - VAPB
  - DCTN1
- Rest is undefined
ALSIN (ALS2)

- ALS, Primary Lateral Sclerosis, Spastic Paralysis
- Ubiquitously expressed and abundant in neurons
- Contains 3 guanine exchange factors (GEFs)
- Function not fully understood
  - Regulating endosomal trafficking
- Has many interacting domains
Motor Proteins and Axonal Transport

(Pasinelli and Brown, 2006, Nature Publishing Group)
Axonal transport dysfunction

• Common molecular pathology in many motor neuron diseases
  ▫ Crucial for the long axons
  ▫ Important components are synthesized in the cell body and carried into the axon

• This makes the possible interactions of motor proteins and Alsin to be the main focus of this study
Aim of the Project
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• Alsin expression changes in
  ▫ differentiated cells
  ▫ undifferentiated cells

• Expression changes of selected genes in Alsin knock-down cells
  • DCTN1 2p13
  • DCTN2 12q13.2-q13.3
  • DCTN3 9p13
  • Kif3a 5q31
  • Kif3b 20q11.21
  • Kif5a 12q13.13

• Possible functions of Alsin
Material and Method
Cell Lines

**N2a [Mouse Neuroblastoma]**
- Isolated from the brain tissue of albino mice
- Cells that have neuron specific characteristics
- Differentiate easily

**NSC34 [Mouse Hybrid cell]**
- Fusion of:
  - Mouse N18TG2 neuroblastoma
  - Embryonic spinal-cord cells
- Cells that express motor neuron characteristics
- Grow and differentiate easily
Sub-culturing

**N2a [Mouse Neuroblastoma]**
Complete MEM medium with
10% FBS, 1% NEAA, 1% Glu, 1%P/S

37°C incubation
Discard old medium
Wash with PBS
Wash cells from the flask with medium,
Centrifuge
Remove supernatant
Resuspend the cells with medium
Add resuspension to new flasks

**NSC34 [Mouse Hybrid cell]**
Complete DMEM medium with
10% FBS, 1% NEAA, 1% Glu, 1%P/S
Differentiation

**N2a [Mouse Neuroblastoma]**

Differentiation medium with
1% FBS, 1% NEAA, 1% Glu, 1%P/S

Add retinoic acid (15uM)

**NSC34 [Mouse Hybrid cell]**

Discard old medium

Complete DMEM-diff medium with
1% FBS, 1% NEAA, 1% Glu, 1%P/S

Complete DMEM-diff medium with
1% FBS, 1% NEAA, 1% Glu, 1%P/S
In vitro transcription

Alsin5a (20pmol/ul) + T7 promoter sequence oligo (20pmol/ul)

2 minutes at 95°C

Cooling at RT

2X T7 buffer, 50mM each rNTP, 100U Ppase, 40U Rnase inhibitor, 100U T7 RNA polymerase + 200pmol DNA template

Incubate at 37°C for 2 hr

Add 1U DNaseI (Rnase free)

Incubate at 37°C for 15 min.

Mix tubes contents

Heat at 95°C for 5 minutes

Incubate at 37°C for 1 hr

Precipitation with NaOAc and EtOH

• Jacquier et al., 2006, Ann Neurol.
• Donze and Picard, 2002, Nucleic Acids Researchs
siRNA Transfection with Roche, X-tremeGENE siRNA transfection reagent

1. Dilute transfection reagent with antibiotic and serum-free medium (1:4)
2. Dilute siRNA with antibiotic and serum-free medium (2ug in 50ul)
3. Mix and incubate at RT (15-20 minutes)
4. Add mixture to the cells
5. Incubate transfected cells (72 hours)
6. GENE KNOCKDOWN
RNA isolation and qRT-PCR

- Total RNA isolation with *Roche High Pure RNA Isolation Kit*
- Primers are adopted from
  - [http://pga.mgh.harvard.edu/primerbank/](http://pga.mgh.harvard.edu/primerbank/)

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Absolute Quantification of Gene Expressions

- Total RNA Isolation
- Reverse Transcription (Using Gene Specific Primers from Harvard Primer Bank)
- Absolute Quantification (Using SYBR Green)
- Data Analysis
Preliminary Results
N2a Cells  [Mouse Neuroblastoma]

Differentiation

Serum deprivation  +  Retinoic Acid (15uM)
NSC34 Cells  [Mouse Hybrid cells]
Expression level is assumed to remain constant
Normalize RNA quantitation in qRT-PCR
HouseKeeping Gene
siRNA Mediated Alsin Knockdown Result on diff. N2a Cells

70.25% decrease in mRNA level
Further Experiments
Further Experiments

• Changes in expression level of selected genes in Alsin KDs does not always indicate a change in their existing protein levels

• Thus it is important to further analyze genes that show difference in expression level by:
  ▫ Immunoprecipitation
  ▫ Westernblot
*THANK YOU FOR YOUR PATIENCE*

Prof. Dr. A. Nazlı BAŞAK