Analysing neurodegenerative gene networks in Amyotrophic Lateral Sclerosis at the single-cell level.

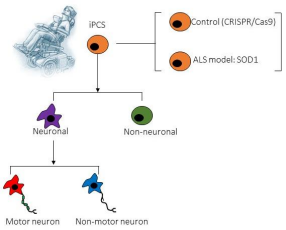
Patricia Thomas, Ryan Ames, Craig Willis, Seema C. Namboori, Akshay Bhinge
University of Exeter Medical School, University of Exeter, UK
Contact: a.bhinge@Exeter.ac.uk

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative condition affecting the spinal motor neurons. The causation and underlying mechanisms of ALS remain unknown. Numerous superoxide dismutase 1 (SOD1) gene mutations have been identified in individuals with ALS. However, the extent to which SOD1 mutations impinge on intracellular pathways, downstream causing motor neuron cell-death, remains unclear.

Research Aim

To identify gene networks which contribute towards motor neuron loss in ALS with a SOD1 mutation, by analysing degenerating neurons at a single-cell level.

Generating isogenic controls and spinal motor neurons

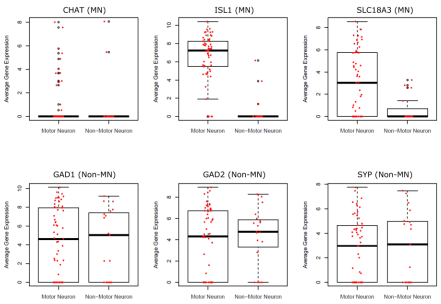


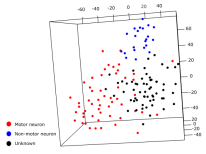
Induced pluripotent stem-cells (iPSC) derived from an individual with ALS bearing the SOD1 mutation E100G, were edited using CRISPR-Cas9 technology to generate isogenic controls.

Both ALS and isogenic control iPSC were differentiated into spinal motor neurons. Single-cell deep RNA-sequencing (RNA-seq) provided an extensive genome-wide transcriptome profile of these neurons.

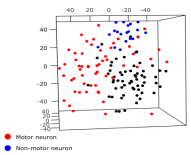
Cell type identification

Using the ALS and isogenic control RNA-Seq data, hierarchical clustering and total stochastic neighbour embedding (tSNE) classified cells into motor neurons and non motor neurons according to their transcriptomic profile. This included performing clustering analyses on cells expressing known motor neuron marker genes (below); identifying further motor neuron classifier genes via differential gene expression and applying a random forest algorithm for cell classification.





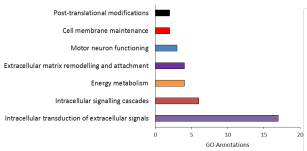
t-Distributed Stochastic Neighbour Embedding (tSNE) of isogenic control neurons



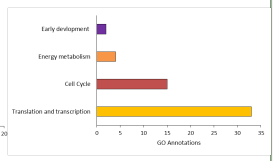
t-Distributed Stochastic Neighbour Embedding (tSNE) of ALS neurons

Differential expression and gene set enrichment analysis

546 genes were identified as being differentially expressed in motor neurons from subjects with ALS compared to isogenic controls. Of these 421 genes were upregulated and 125 downregulated. Gene set enrichment analysis (GSEA) identified signal transduction, mRNA and energy metabolism genes to be dysregulated in ALS motor neurons.

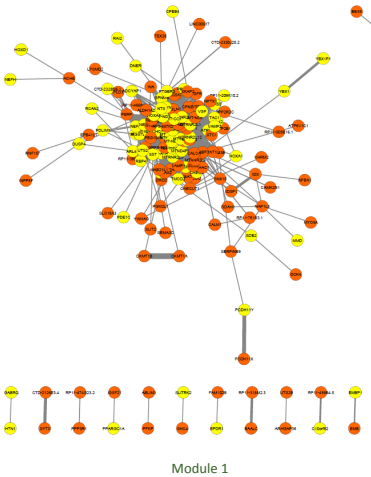


GSEA upregulated genes in ALS

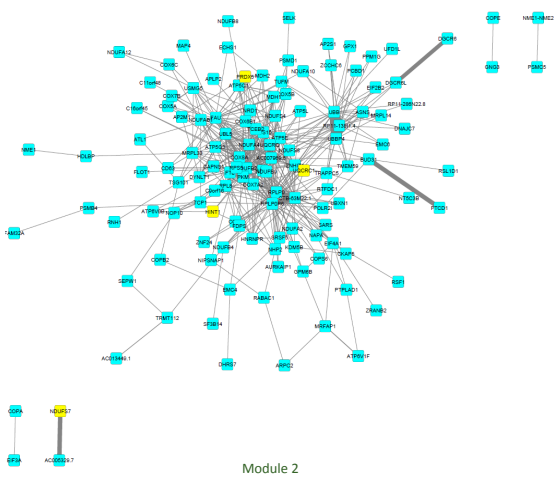


GSEA downregulated genes in ALS

Gene co-expression networks



Module 1



Module 2

Gene co-expression networks were constructed using weighted gene correlation network analysis (WGCNA). Points represent the genes in the network, and edges the gene-gene interactions. Points in yellow represent those genes differentially expressed in ALS motor neurons compared to isogenic controls.

Module one (orange above) and two (blue above) were deemed to be downregulated ($p<0.05$) in ALS motor neurons, and have a gene ontology of regulating the ribonucleotide metabolic processes (module 1), oxidative phosphorylation (module 1), and mitochondrial ATP synthesis coupled to electron transport (module 2).

Conclusion

Energy metabolism is a core network disrupted in mutant SOD1 bearing motor neurons and may contribute towards neurodegeneration in ALS.

References

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