Axonal transcriptome of stem cell-derived motor neurons in health and ALS

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Background

Spinal motor neurons are highly polarised cells. Their cell bodies are located in the spinal cord, while their axons can span up to a meter to connect with muscle at the neuromuscular junction (NMJ). High metabolic demand and the presence of ribosomes indicate local translation of mRNA. To uncover the axonal transcriptome of stem cell-derived motor axons we combined microfluidics with RNA sequencing. **Axon retraction from the NMJ** is the first pathological symptom in amyotrophic lateral sclerosis (ALS). Investigating the axonal transcriptome can therefore have implications for research on motor neuron degeneration in ALS. We used SOD1^{G93A} over-expressing stem cell-derived motor neurons as a model for ALS and found that their axonal transcriptome differs from control axons.

Aims

- Culture spinal motor neurons derived from mouse embryonic stem cells in microfluidic devices to separate somas and axons.
- Perform RNA sequencing on somas and axons separately.
- Analyze transcriptional changes induced by mutant SOD1^{G93A} in motor axons.

Microfluidic culture platform

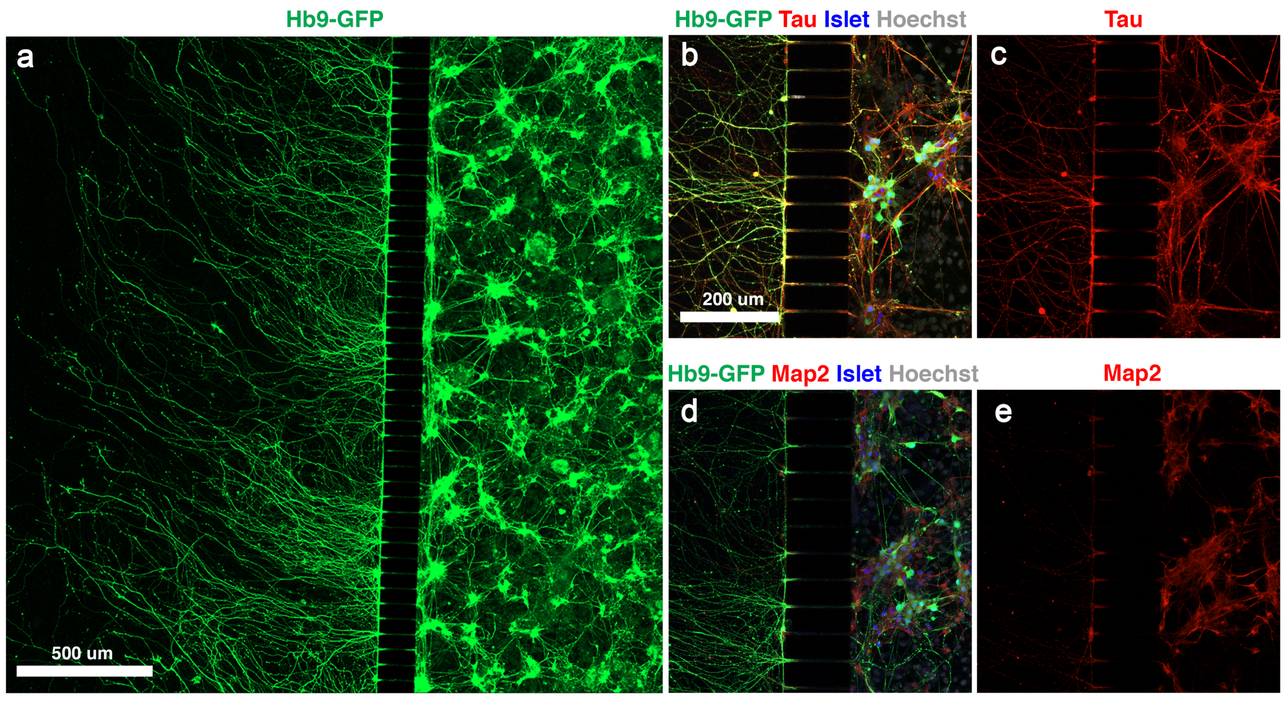

Fig. 1 (a) Hb9-GFP positive mESC-derived motor neurons[1] cultured in a microfluidic device. Axons can grow through 3 micron high microgrooves. The somas cannot cross these grooves. **(b,c)** Tau was expressed in the processes that extended to the axonal compartment. **(d,e)** Map2 was not strongly expressed in the axonal compartment, indicating that relatively few dendrites cross the grooves.

Axonal RNA can be selectively obtained

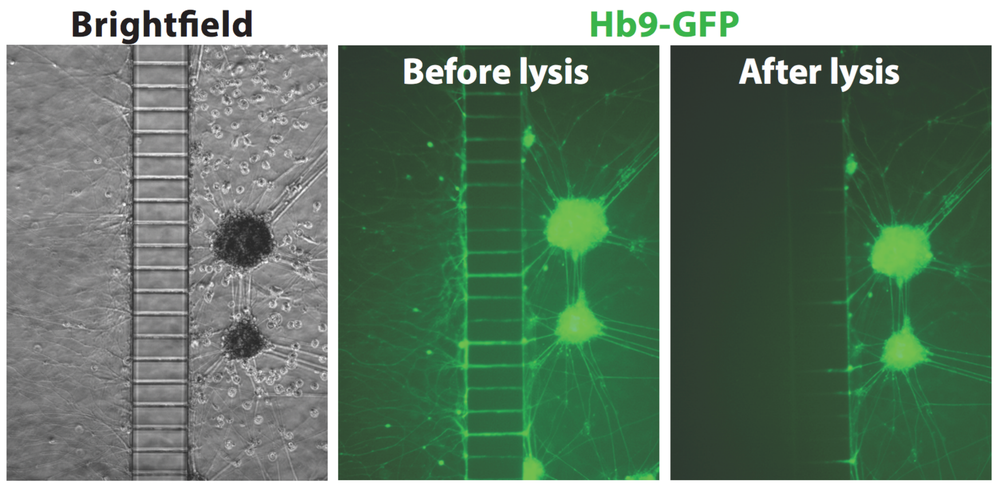

Fig. 2 Axons can be separately lysed without affecting the somal compartment, allowing for the sampling of axonal and somal RNA. Quality control was performed afterwards to exclude axonal samples that were 'contaminated' with somas.

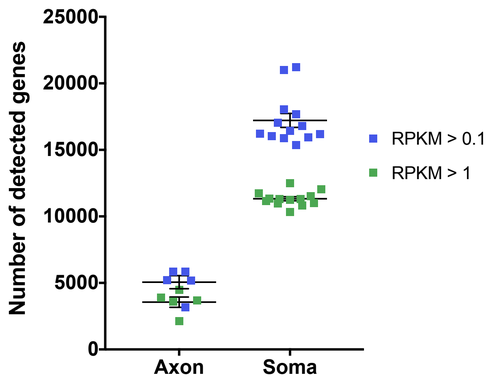

Fig. 3 Detected number of transcripts in motor neuron somas and axons at >1 and >0.1 RPKM (reads per kilobase million). In somas around 12,000 transcripts were detected at >1 RPKM and an additional 5,000 more lower abundant transcripts at RPKM >0.1. Axons contained around 4,000 transcripts at RPKM >1 and an additional 1,500 at RPKM >0.1.

Motor axons have a distinct transcriptome

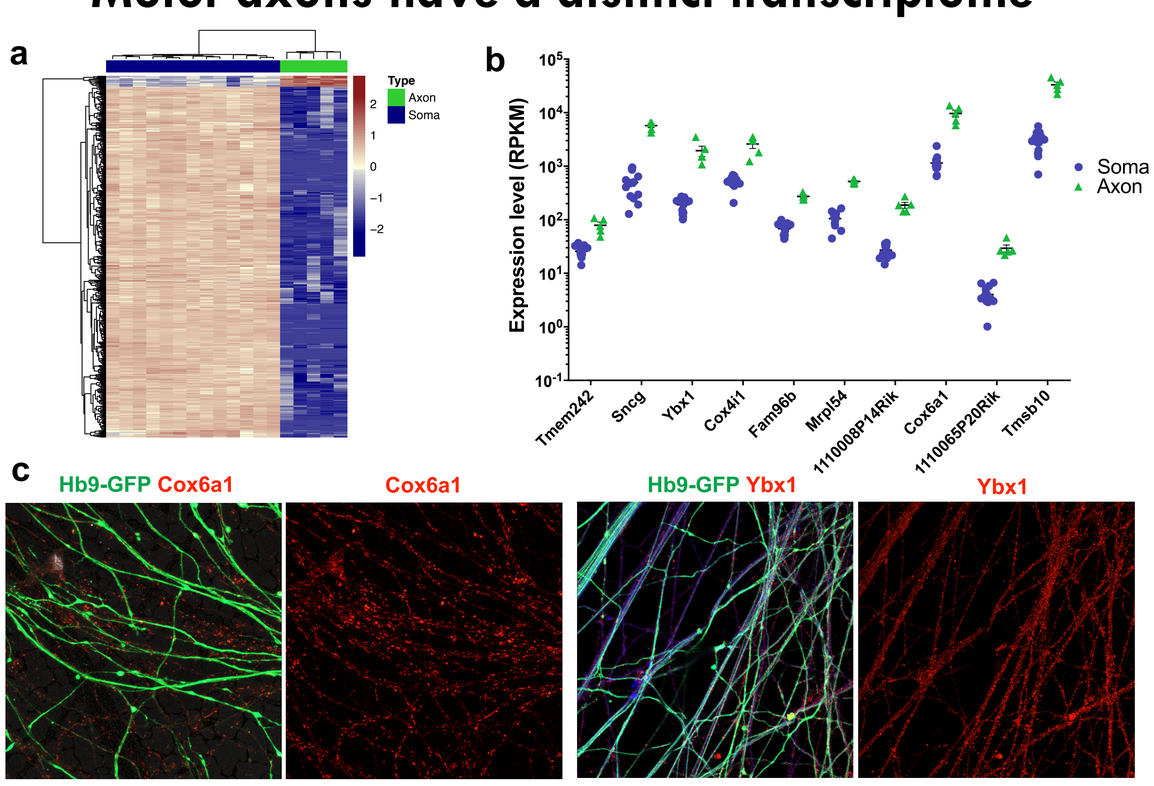

Fig. 4 Analysis of the axonal versus the somal transcriptome in control motor neurons (a) Heatmap of the top 500 differentially expressed genes (adjusted p < 0.05) between somas and axons in control motor neurons. **In total, 660 genes were enriched in the axonal fraction. (b)** Top 10 axonal-enriched genes by adjusted p-value. **(c)** Immunocytochemistry for two axonally enriched genes, showing their presence at the protein level in axons. Cox6a1 is a component of the mitochondrial respiratory chain. Ybx1 is an mRNA-binding protein known to mediate alternative splicing of pre-mRNAs.

Transcriptome differences between control and SOD1^{G93A} axons

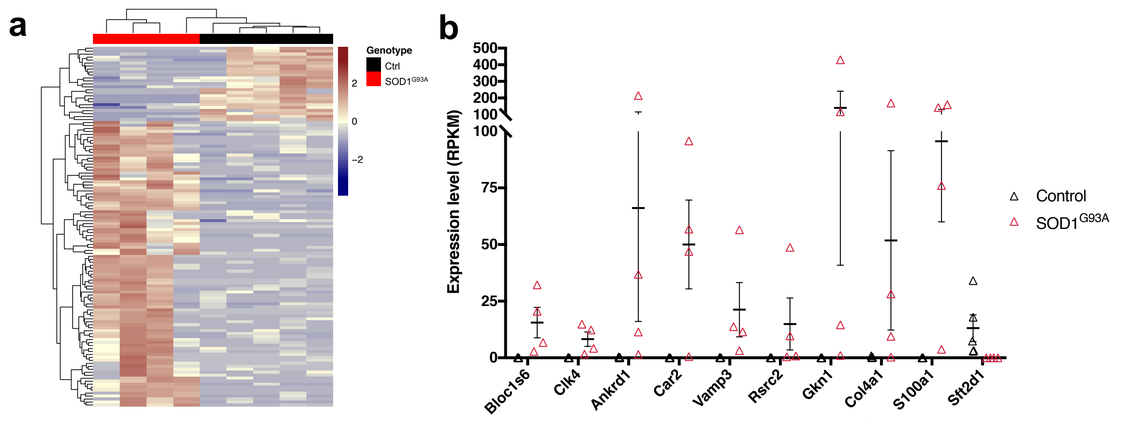

Fig. 5 Analysis of the axonal transcriptomes of control and SOD1^{G93A} overexpressing motor neurons (a) Differentially expressed genes between control and SOD1^{G93A} axons. In total, 121 genes were differentially expressed, of which **96 genes were enriched in SOD1^{G93A} axons and 25 were enriched in control axons. (b)** Top 10 differentially expressed genes by adjusted p-value.

Conclusions

We demonstrate that the transcriptome of motor axons can be selectively investigated using microfluidics coupled with RNA sequencing. Distal motor axons show a distinct expression pattern compared with somas, with more than 5,000 transcripts detected in axons. Comparison with SOD1^{G93A} overexpressing motor neurons showed that their axonal mRNA composition differs from control axons. Since motor axon retraction from muscle is the primary pathological event in ALS, these transcriptome differences can provides clues to the mechanisms of vulnerability of the distal axon.

References

1. Wichterle H, Lieberam I, Porter J, Jessell T. *Cell* 2002; 110.

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