7SK depletion preserves the somatic transcriptome but affects axonal RNA homeostasis in human iPSC-derived motoneurons

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Background: The 7SK small nuclear ribonucleoprotein (7SK snRNP) complex is a key regulator of transcriptional elongation through its control of the P-TEFb complex, composed of CDK9 and Cyclin T1. While its function has been extensively characterized in dividing cells, its role in highly polarized cells such as motoneurons remains largely unexplored. We investigated the consequences of 7SK depletion in human iPSC-derived motoneurons to determine its role in neuronal RNA homeostasis. Method: 7SK was depleted using gapmer-based antisense oligonucleotides (ASOs) in human iPSCderived motoneurons cultured in compartmentalized microfluidic chambers, allowing the separation of axons from cell bodies for compartment-specific RNA sequencing. Transcriptomic changes were analyzed by RNA-seq, and the expression of 7SK snRNP components was assessed at both the RNA and protein levels. To validate the findings in vivo, a 7SK knockout mouse model was generated and analyzed. Results: ASO treatment reduced 7SK RNA levels by approximately 80%, enabling efficient depletion for downstream analyses. RNA-seq revealed no significant changes in the somatic transcriptome following 7SK depletion, indicating that steady-state gene expression in neuronal cell bodies is largely preserved. In contrast, the axonal transcriptome was profoundly altered, demonstrating a critical role for 7SK in maintaining axonal RNA composition. Although mRNA levels of 7SK snRNP components remained unchanged, the abundance of their corresponding proteins was markedly reduced, suggesting posttranscriptional compensation in response to 7SK depletion. Similar molecular alterations were observed in the mice model, indicating that these effects are conserved across human and mouse motoneuron systems. Conclusion: Our findings demonstrate that the primary consequence of 7SK deficiency is not widespread transcriptional dysregulation in neuronal soma but rather disruption of the axonal transcriptome, leading to compartment-specific RNA dyshomeostasis. These results identify a previously unrecognized role for 7SK in maintaining axonal RNA composition and provide new insights into the molecular mechanisms that preserve motoneuron integrity