Examining the Effect of TBC1D7 Depletion on Neuronal Migration and Organization in the Developing Cerebral Cortex

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Abstract

Tuberous sclerosis (TS) is an autosomal dominant disorder caused by a mutation in either the TSC1 or TSC2 gene of the tuberous sclerosis complex (TSC). It has been shown that human TSC1 and TSC2 mutations alter neural shape, organization, and complexity (Bassetti et al., 2021). In addition to TSC1 and TSC2, the TS complex also includes TBC1D7. However, the functions of TBC1D7 and how it affects brain formation remain largely unknown.

Here, we examined the effect of TBC1D7 depletion on neuronal migration and organization in the developing brain.

We found that TBC1D7 knockdown leads to disrupted neuronal migration and distribution of TBC1D7 deficient neurons. Our results suggest that TBC1D7 depletion further compounds the TSC1/2 deficient phenotype by disrupting neuronal migration, altering cortical lamination, thus leading to ectopic accumulation of cortical neurons in the SVZ and IZ. These studies highlight the importance of TBC1D7 in the stabilization of the TS complex and help link human brain malformations seen in TSC to disruptions in specific cortical embryonic development events such as neuronal migration and laminar organization.

Introduction

TBC1D7 is a ubiquitous core component of the TS complex. However, little is known about the function of TBC1D7 and how it affects brain formation.

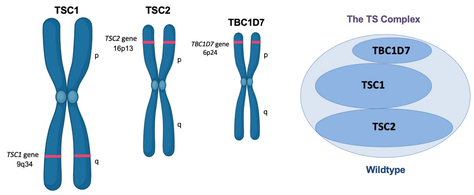


Figure 1. The TS complex is made up of TSC1, TSC2, and TBC1D7.

The goal of our experiment is to determine the effect of TBC1D7 depletion on neuronal placement and organization in the developing brain. We aim to test if TBC1D7 is a functionally essential component of the TS complex and the critical role it plays in the development of the cerebral cortex.

Methods and Materials

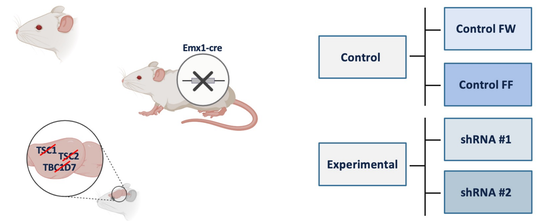


Figure 2. Mouse models and experimental set up. We used TSC deficient mouse models and shRNA-based gene knockdown approaches. Emx1-cre was used to deplete TSC1/2 in the developing cerebral cortex. TBC1D7-specific shRNAs were used to knockdown TBC1D7 in TSC1/2 null brains.

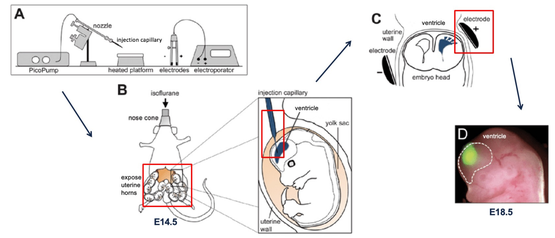


Figure 3. *In utero* electroporation. (**A-C**) *In utero* electroporation (IUE) was performed on pregnant mice at E14.5. (**D**) Embryos were collected at E18.5 for analysis. Scheme adapted from Artegiani et al., 2016.

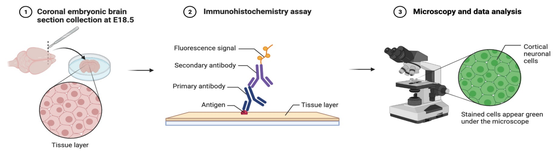


Figure 4. Immunohistochemistry (IHC). Embryonic brains were collected at E18.5, sectioned coronally, and processed for immunohistochemical detection of TBC1D7 deficient neurons. Images were visualized using a confocal laser scanning microscope.

Results

- Altered migration and distribution of GFP$^+$/ TBC1D7 cells throughout the developing cerebral wall following TBC1D7 knockdown in TSC1/2 mutants (**Fig. 5; Fig. 6**).
- Depletion of TBC1D7 in the TSC1/2 mutants led to further disruption of TSC1/2 phenotype (**Fig. 6**).
- In addition to the expansion of the upper layer neurons and altered cortical lamination seen in the TSC1/2 mutant phenotypes, TBC1D7 knockdown led to ectopic accumulation of cortical neurons in the SVZ/IZ (**Fig. 7**).

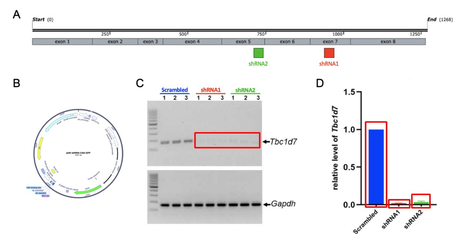


Figure 5. TBC1D7 was successfully knocked down in shRNA #1 and shRNA #2.

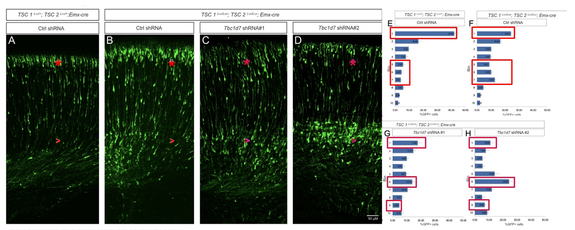


Figure 6. TBC1D7 knockdown leads to an altered distribution of GFP$^+$/ TBC1D7 neuronal cells in the developing cortex.

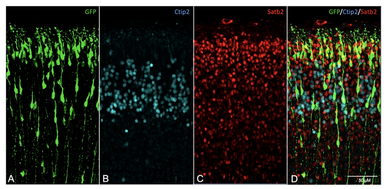
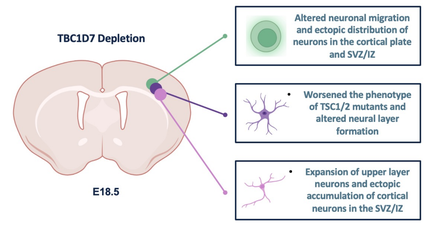


Figure 7. Assay to identify whether the absence of TBC1D7 altered the number and type of cortical neurons.

Discussion and Conclusions

Neuronal migration and distribution of neurons in the developing cerebral wall were altered when TBC1D7 was depleted (**Fig. 8**).

TBC1D7 may serve as a scaffold for protein-protein interactions and stabilize TS complex (Madigan et al., 2018). TBC1D7 knockdown decreases the association of TSC1 and TSC2 leading to increased mTORC1 signaling (Dibble et al., 2012), suggesting that TBC1D7 is important in the stabilization of the TS complex. Inactivating TBC1D7 disrupts the TS complex, which may underlie the altered neuronal migration and distribution observed.



In addition to altered distribution of neurons, TBC1D7 knockdown may also have influenced neurogenesis. Quantification of deep and upper layer neuronal cell density following TBC1D7 knockdown (e.g.; **Fig. 7**) will be necessary to fully understand the role of TBC1D7 depletion in abnormal neural development.

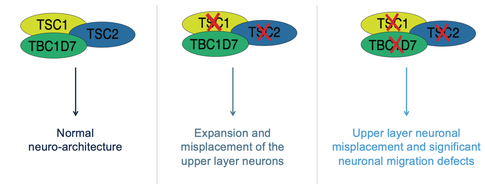


Figure 8. Schematic representation of conclusions following TBC1D7 knockdown in TSC1/2 null cortices.

Further, exploring brain malformations in TSC will allow us to analyze the functional pathways that regulate normal development and organizations of neurons.

By characterizing the functions of TBC1D7, these new observations may help link human brain malformations seen in TSC to disruptions in cortical development events, such as neurogenesis and neuronal migration.

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