Slc7a5 regulation of neural development

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Membrane transport proteins are appreciated for their ability to transport molecules across biological membranes and have received a renewed focus for their role in nervous system disorders. One such group of transporters are members of the solute carrier (SLC) family. SLC transporters shuttle various molecules, including neurotransmitters, fatty acids, amino acids, and inorganic ions. As such, they play an integral role in supplying cells with essential resources. An example is that amino acids are transported by SLC family members. Slc7a5 is a SLC family member that transports essential amino acids important for neuronal development, as well as other biological molecules including thyroid hormones T3, T4, and L-Dopa. Slc7a5 forms a heterodimer with Slc3a2 that facilitates the subcellular distribution of Slc7a5. Amino acids including leucine, glutamine, and arginine support cell growth by activating the protein kinase, mammalian target of rapamycin complex 1 (mTORC1) (Liu et al., 2020). Consistent with this notion is that Slc7a5 transport of amino acids regulates mTORC1 activity in numerous cell types (Nicklin et al., 2009). A recent manuscript expands upon these findings to demonstrate that Slc7a5 plays a previously unrecognized and critical role in the regulation of mTORC1 activity and neuron development (Sokolov et al., 2020).

Pathogenic mutations in *SLC7A5* that prevent efficient amino acid transport are correlated with delayed motor, social, and language development (Tărlungeanu et al., 2016). Patients with *SLC7A5* mutations may also present with autism associated disorder and seizures (Tărlungeanu et al., 2016). Although it is unclear whether neurological manifestations are caused by changes in the periphery, MRI demonstrates microcephaly and a thinning of the corpus callosum (Tărlungeanu et al., 2016). Thus, one might assume that Slc7a5 has a direct role in brain development. However, Slc7a5 is highly expressed within endothelial cells of the blood-brain barrier (BBB), but is curiously undetectable in neurons in the adult brain (Tărlungeanu et al., 2016). Thus, Slc7a5 may have an important role in regulating essential amino acid influx into the brain. In agreement, *Slc7a5* deletion from endothelial cells prevents postnatal leucine and isoleucine import in to and histidine export out of the brain (Tărlungeanu et al., 2016). Loss of Slc7a5 from endothelial cells in transgenic mice causes kyphosis and fine motor and locomotor defects (Tărlungeanu et al., 2016). Arguably more provocative is the neurobehavioral manifestations caused by loss of Slc7a5 from endothelial cells in transgenic mice. This includes reduced socialization, no preference between novel objects and conspecifics, and increased isolation-induced ultrasonic vocalizations, all associated with autism-like manifestations (Tărlungeanu et al., 2016). One mechanism for behavioral manifestations is that endothelial *Slc7a5* deletion reduces the frequency of miniature inhibitory synaptic currents in glutamatergic pyramidal neurons of the cerebral cortex (Tărlungeanu et al., 2016). Thus, Slc7a5 allows the BBB to transport amino acids to the brain that are required for neuron function. However, additional cell types in the brain might also require Slc7a5. In agreement, *Slc7a5* mRNA is also present in developing mouse embryos, specifically in early structures of the developing nervous system including the neural plate prior to maturation of a BBB (Poncet et al., 2020).

The importance of Slc7a5 is further highlighted by the fact that knockout is lethal by embryonic day (e)11.5 in mice (Poncet et al., 2020). *Slc7a5* knockout embryonic mice exhibit neural tube closure defects and often fail to expand the forebrain (Poncet et al., 2020). The lack of forebrain outgrowth following *Slc7a5* deletion phenocopies embryos with reduced mammalian target of rapamycin (mTOR) levels caused by mTOR mutations, knockout, or pharmacological inhibition with rapamycin, and develop a "flat-top" structure (Hentges et al., 2001). In further support of this link, *Slc7a5* knockout embryos have reduced mTOR pathway activity within the developing nervous system (Poncet et al., 2020). Consistent with mTORs prominent role in nervous system development, *Slc7a5* knockout embryos have less NeuroG2, a transcription factor that drives neural progenitors to exit the cell cycle and differentiate (Poncet et al., 2020). Not surprisingly, *Slc7a5* knockout embryos have reduced βIII-tubulin indicative of fewer neurons and defective axon tract (Poncet et al., 2020). The loss of neurons is likely caused by loss of amino acid transport that causes an integrative stress response and an increase in apoptosis (Poncet et al., 2020). This data provides compelling evidence that Slc7a5 is required for nervous system development and further support a link to mTOR. However, the use of knockout mice did not address which cells require Slc7a5 for neural development.

To address this limitation, a recent manuscript examined the role of Slc7a5 in neural development in the context of neonatal subventricular zone (SVZ) neurogenesis and the production of olfactory bulb (OB) neurons (Sokolov et al., 2020). The SVZ is a neurogenic region that surrounds the lateral ventricles of the brain. Neural stem cells (NSCs) that reside in the SVZ generate progenitors that produce immature neurons called neuroblasts. Neuroblasts migrate through the rostral migratory stream and then radially before settling in the granule cell (GC) layer of the OB. Neuroblasts predominantly mature into axonless GABAergic inhibitory GCs. GC basal dendrites develop connections with neighboring GCs, while an apical dendrite extends out toward the external plexiform layer before branching. Between 14 and 30 days after the birth date of a GC, the apical dendrites continue to elaborate and form reciprocal dendrodendritic synapses with mitral/tufted cells. Mitral/tufted cells release glutamate onto GC dendrites and GCs subsequently release GABA onto mitral/tufted cell dendrites to mediate lateral inhibition and regulate olfaction (**Figure 1A**).

Neonatal SVZ electroporation was performed to alter Slc7a5 *in vivo* (Sokolov et al., 2020). Neonatal SVZ electroporation is a technique for which plasmid DNA is introduced into the lateral ventricles of the neonatal mouse brain followed by application of a small electrical field. Neonatal electroporation allows the introduction of plasmid DNA into SVZ NSCs. Since NSCs pass plasmids to daughter cells, the neuroblasts that eventually mature into GCs retain and express the encoded content. Moreover, the dilution of plasmid DNA also conveniently assigns approximate birth dates to labeled GCs. Plasmid DNA encoding fluorescent marker proteins to visualize cells and short hairpin RNA (shRNA) to induce RNA interference of *Slc7a5* were electroporated into SVZ NSCs (Sokolov et al., 2020). An additional cohort of mice were electroporated with a plasmid encoding human *SLC7A5* to overexpress SLC7A5 (Sokolov et al., 2020). No developmental defects were noted when SLC7A5 was overexpressed, which could be due to the fact that the rate limiting step for Slc7a5 mediated mTORC1 activation was shown in a previous study to be the efflux of glutamine (Nicklin et al., 2009). Neuroblasts in all conditions migrated to the OB and initiated dendrite outgrowth that was unaltered 14 days after electroporation (Sokolov et al., 2020). However, Slc7a5 knockdown caused a dramatic loss of dendrites 30 days after electroporation (Sokolov et al., 2020). This stunted dendrite morphology was accompanied by a significant loss of GCs (**Figure 1B**; Sokolov et al., 2020).

GC dendrite arborization is highly dependent on mTORC1 (Skalecka et al., 2016). Slc7a5 transport of leucine has been proposed to regulate mTORC1 pathway in cultured cortical neurons (Ishizuka et al., 2008). Based on these facts, the loss of dendrites in Slc7a5 knockdown GCs was hypothesized to be mediated by mTORC1. Sokolov et al. (2020) confirmed that Slc7a5 knockdown decreased mTORC1 pathway activity, and

that Rheb overexpression rescued mTORC1 pathway activity. Activation of the mTORC1 pathway was associated with a rescue of dendrite complexity and a partial rescue of GC survival (**Figure 1C**; Sokolov et al., 2020). The significant rescue in dendrite complexity compared to cell number indicates that the two manifestations of Slc7a5 knockdown may be separable, with stunted dendrite arbors being a primary effect and loss of cells secondary. Although ectopic Rheb expression corrected mTORC1 pathway activity which supports dendrite arbors, the lack of amino acids caused by Slc7a5 knockdown might be unsustainable for GC survival. It is also worth noting that the accumulation and storage of amino acids may be an ongoing process starting in NSCs. The effect on GCs could reach a critical threshold between 14–30 days after Slc7a5 knockdown, at which point amino acid depletion causes defects.

Several alternative mechanisms could be responsible for loss-of-Slc7a5 phenotypes. One alternative explanation is that Slc7a5 knockdown leads to aberrant Kv1.2 regulation (Baronas et al., 2018). Kv1.2 is a voltage-dependent delayed rectifier potassium channel that promotes repolarization after action potentials. Slc7a5 physically interacts with and regulates Kv1.2. Slc7a5 co-expression reduces Kv1.2 protein expression, accelerates Kv1.2 inactivation, and hyperpolarizes Kv1.2 activation. Slc7a5 likely functions in an analogous manner as the canonical accessory subunit of Kv1.2, Kvβ (Lamothe and Kurata, 2020). While these studies were predominantly performed in non-neuronal cells *in vitro*, Slc7a5 and Kv1.2 were detected in cortical and hippocampal neurons (Baronas et al., 2018). Kv1.2 knockout mice do not survive past 3 weeks due to seizures (Brew et al., 2007). Thus, a complementary mechanism is that loss of Slc7a5 could lead to changes in Kv1.2 that effect GC morphology and survival.

Another consideration is that Slc7a5 can transport L-Dopa which is the precursor to the neurotransmitter dopamine. Down-regulation of Slc7a5-like transporters LAT1-like transporters juvenile hormone inducible-21 and minidiscs, in mature Drosophila dopaminergic neurons disturbs sleep patterns in Drosophila (Aboudhiaf et al., 2018). In part, this is mediated by changes in sensitivity to L-Dopa although brain dopamine levels do not change (Aboudhiaf et al., 2018). However, sleep pattern defects following loss of juvenile hormone inducible-21 can be rescued by Rheb and confirm the critical evolutionary link between amino acid transporters in the brain and Tor (Aboudhiaf et al., 2018). Consistent with the effect of Slc7a5 being independent of L-Dopa, Slc7a5-null mouse embryos have negligible reductions in dopamine regulated gene expression (Poncet et al., 2020).

Taken together, Slc7a5 is required for proper brain development and the most recent additions to the growing body of Slc7a5 literature provides a narrative for understanding how amino acid transporters differentially regulate diverse cell types and distinct cellular processes in the nervous system.

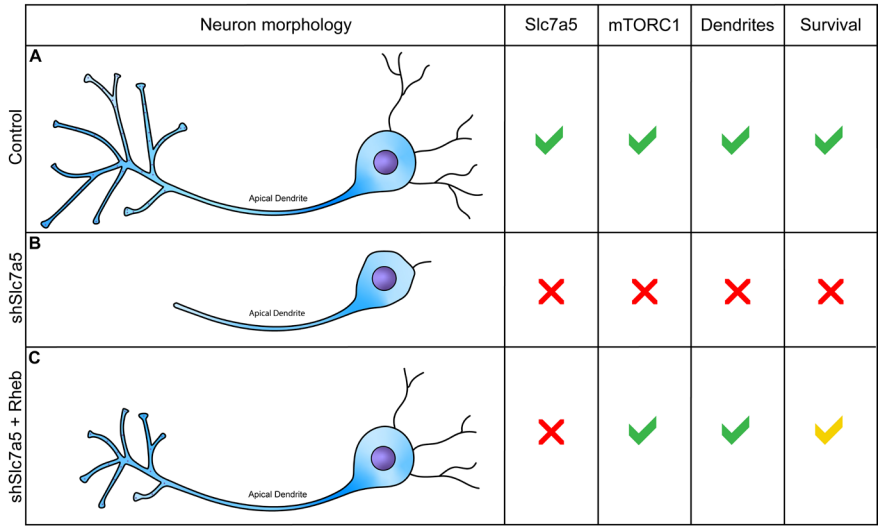

Figure 1 | **Slc7a5 regulation of mTORC1, dendrite morphology, and survival.**
Slc7a5 is an essential amino acid transporter that regulates the mTORC1 pathway. Neural stem cells of the subventricular zone produce neuroblasts that migrate along the rostral migratory stream into the GC layer of the olfactory bulb. Neuroblasts begin to mature into GCs by 7 days after their birthdate. GC apical dendrites project into the external plexiform layer 14 days after the birthdate. (A) Post-natal day (P) 30 GCs have undergone extensive basal and apical dendrite outgrowth. (B) mTORC1 pathway activity is decreased, dendrite arbors are reduced, and fewer GCs remain at P30 following Slc7a5 knockdown. (C) Rheb co-expression rescues dendrite arbors in Slc7a5 knockdown GCs. GC: Granule cell; mTORC1: mammalian target of rapamycin complex 1.

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