A patient-derived iPSC model to study glutamate deficiency by SHANK-3 mutation in Autism Spectrum Disorder

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BACKGROUND

The SHANK gene family encodes for synaptic scaffolding proteins enriched in the postsynaptic density of excitatory synapses, and plays important roles in the formation, maturation, and maintenance of synapses. A silent mutation of *SHANK-3* is estimated to be carried by 0.5-1.5% persons with Autism Spectrum Disorder (ASD). It is integral to understand how the *SHANK-3* mutation plays a role in regulating glutamate levels and the role of medications that treat ASD, such as riluzole, and other neurological diseases. As a model of the forebrain, neuron-astrocyte co-cultures were developed from human pluripotent stem cells. Cells used were a from patient line containing a *SHANK-3* mutation, as well as a CRISPR-corrected control. For this experiment, co-cultures were treated for 24 hours with the drug riluzole in varying concentrations (0.5μM, 1μM) and secreted glutamate levels were measured through a glutamate ELISA assay (Figure 3). qPCR was used to validate the presence of mature neurons and astrocytes in the co-cultures after differentiation and maturation (Figure 4). Immunofluorescence was also used to quantify the number of synapses in each group via co-localized staining of synaptic markers (Figure 5). The role of the SHANK-3 mutation's effect of glutamate is pertinent to understanding neuropsychological disorders effected by abnormal glutamate levels in the cortex.

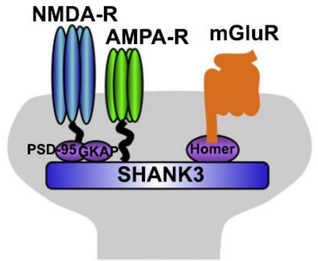


Figure 1: Role of SHANK-3 in glutamate signaling (Uchino & Waga, 2013).

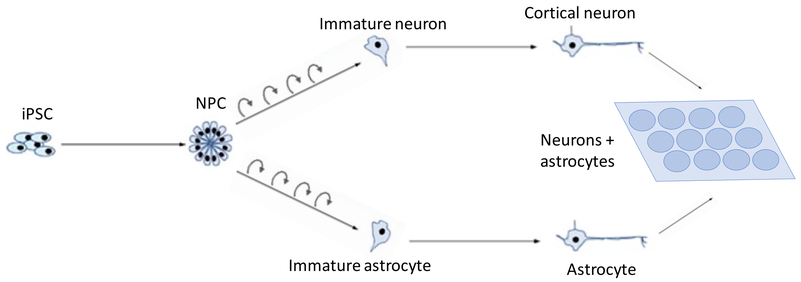
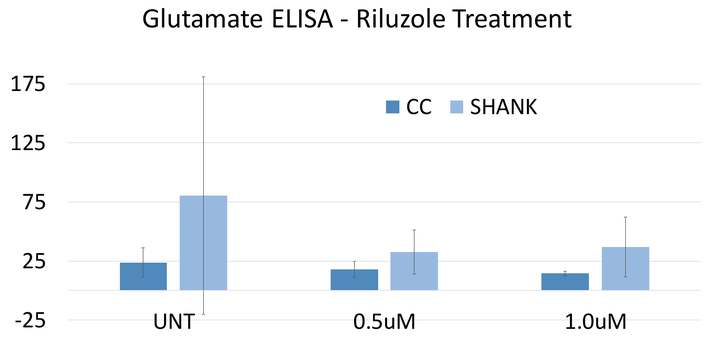
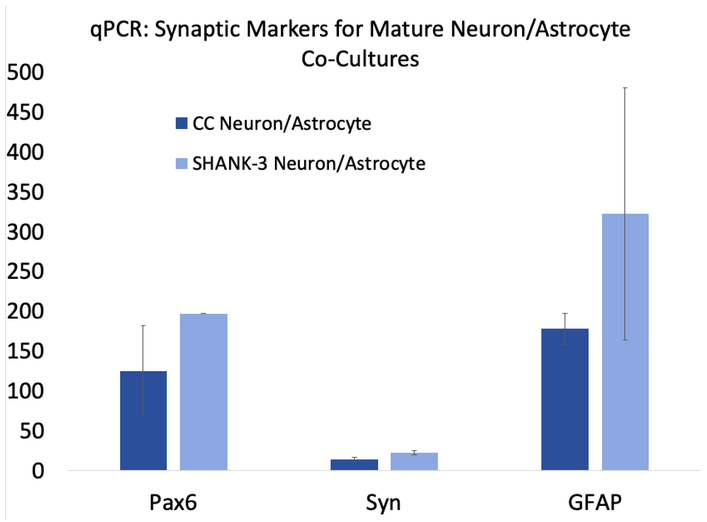


Figure 2: Depiction of protocol to derive neuron/astrocyte co-cultures from patient iPSCs (Darville et al., 2016)

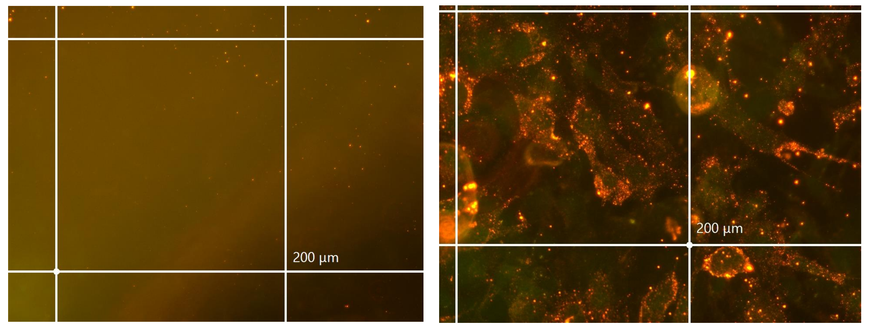
Figure 3 (right): ELISA Analysis of secreted glutamate levels following a 24-hour treatment with 0.5μM or 1μM riluzole. Normal glutamate levels were expressed in the control (CC) and decreased with increasing concentration of riluzole. The *SHANK3* cells expressed more glutamate compared to the control and decreased in a similar fashion with riluzole. Thus, riluzole lowered glutamate in *SHANK3* neuron/astrocyte cocultures closer to normotypic expression.





	Pax6	Syn	GFAP
Control	125	13.8	178
SHANK-3	197	22.5	322

Figure. 4: qPCR analysis of neuron (Pax6/Syn) and astrocyte (GFAP) markers in neuron and astrocyte cocultures. Marker expression are determined as relative to levels in iPSC controls.



A: Control B: *SHANK-3*

	Control	*SHANK-3*
Synapses per well	1.43x10^6	1.28x10^6

Figure 5: Immunofluorescence analysis of neuron/astrocyte co-cultures at day 120. A: Control cell line. B: *SHANK-3* cell line. Images were obtained and analyzed from 12-well co-cultures stained with SV2 (pre-synaptic) and PSD93 (post-synaptic) using a Keyence BZX microscope. Overlapping colocalization signals indicate a synapse and are expressed as bright puncta. Puncta per square area were quantified using ImageJ.

DISCUSSION

Generated from patient iPSCs, the *SHANK-3* mutant neuro/astrocyte co-cultures was predicted to have an abnormal level of synapses and excess glutamate released. qPCR analysis validated that mature neuron/astrocyte co-cultures were grown from iPSC lines. Immunofluorescence revealed a decrease in the number of synapses between the *SHANK3* cultures and controls. The ELISA assay shows higher levels of glutamate secreted in the *SHANK-3* cells compared to the controls. Following the treatment of riluzole, glutamate levels in the *SHANK3* cells demonstrated a similar decrease as compared to the control. This experiment emphasizes the importance of normal glutamate levels to maintain neurotypic synaptic activity and the potential role of psychiatric medications as a mediator of aberrant glutamate signaling as a treatment option for ASD.

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