Microelectrode array (MEA) for assessing neuronal network restoration in PTEN knockdown primary hippocampal neurons

PTEN RESEARCH

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Abstract

PTEN hamartoma tumour syndrome (PHTS) is a rare disease (incidence 1:200,000) arising from germline mutations in PTEN. Approximately 25% of people with PHTS meet diagnostic criteria of autism spectrum disorder (ASD) [1] and ~2% of individuals with ASD may harbour a germline PTEN mutation [2]. Such PTEN mutations lead to an upregulation of PI3K-Akt-mTOR signaling that has been associated with macrocephaly and structural and functional changes in hippocampal and cortical neurons in both human and murine CNS. Animal models with PTEN loss show neuronal hyperexcitability and seizure development. Our PTEN knockdown (PTEN KD) hippocampal neuron cell model showed upregulation of PI3K-Akt-mTOR signaling. Micro-electrode array (MEA) in vitro assays offer valuable insights into cellular behavior, particularly in neurons, by measuring electrical activity noninvasively across a cell population over time using electrodes. We showed that AAV shRNA mediated PTEN KD neurons exhibit a consistent increase (e.g., burst duration and network burst duration) and decrease (network burst frequency) over time for a subset of neuronal network parameters linked to neuronal excitability. Rapamycin, an inhibitor of mTOR restored these functional parameters in PTEN KD cells in the direction of non-target shRNA control cells. A structured statistical framework was applied to evaluate the activity of Rapamycin, accounting for experimental variability and potential neurotoxicity over time. Applying the MEA assay to complement other measures of PTEN loss in neurons provides data that may further discriminate between inhibitors of PI3K signaling as drug repurposing candidates.

Introduction

The PTEN phosphatase and PI3K signaling play a significant role in neuron development and function. PI3K pathway components are intimately linked with neuron stem cell growth, dendritic outgrowth and branching, and neurotransmitter release. Germline mutations in PTEN were identified as the causative agent for a number of clinical syndromes now collectively known as the PTEN hamartoma tumor syndrome (PHTS), and the PI3K pathway appears to be a point of convergence for a number of genetic syndromes having autism spectrum disorder (ASD) as a component, including Fragile X, TSC and Rett. In PHTS PTEN loss of function mutations lead to an upregulation of PI3K signaling that is manifest as macrocephaly which has significant penetrance, and as ASD in ~25% individuals. In animal models, loss of PTEN leads to an increased cortical thickness, enhanced neurite growth and branching, dysregulates synaptic plasticity characterized by changes in long-term potentiation and long-term depression [3].

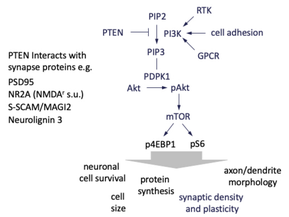


Figure 1. Schematic of PI3K signalling in neurons. PI3K kinases have been associated with specific aspects of CNS function including neuron growth, dendritic growth and branching. PTEN, the negative regulator of PI3K, also interacts directly with various proteins to further influence neuronal activity.

PI3K signalling has been shown to regulate receptor expression and neurotransmitter release and PTEN may have a scaffolding role at the synapse through protein-protein interactions with PDZ domain containing proteins e.g. PSD95 and NMDA receptors [4]. In animal models, knockout of PTEN expression in cortical or dentate gyrus neurons leads to enhanced synaptic activity and we have followed neuronal activity in PTEN loss primary hippocampal neurons as a basis for characterising candidate drug repurposing molecules.

Cellular model: Mouse hippocampal neurons transduced with shRNA targeting PTEN

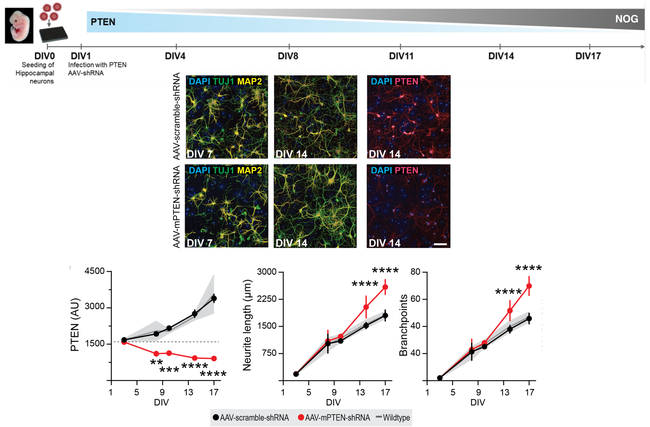


Figure 2. Hippocampal neurons were isolated from embryonic day 16 mouse embryos. Twenty-four hours after plating cells were transduced with an adeno-associated virus expressing a shRNA targeting PTEN (AAV-mPTEN-shRNA), fixed at different time points and stained for image analysis. PTEN KD resulted in PTEN loss robustly at DIV7 accompanied by increase of neurite length and branching at DIV14 and DIV17. Neurite outgrowth (NOG) was assessed with antibodies against Tuj1 combined with MAP2 and DAPI staining for neuronal detection. Mean ± SD are shown (two-way ANOVA, **p<0.01, ***p<0.001, ****p<0.0001). Scale bar 100 µm.

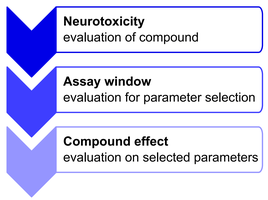
Workflow for biostatistics analysis of MEA data

MEA parameters were analyzed through a workflow of three main points, after extreme outlier removal using Tukey's method.

The neurotoxicity of the compound was evaluated based on mean firing rate after compound administration.

Then, a linear mixed effects model was applied pooling the control treatment data (DMSO) from all experiments, and the assay window between the PTEN KD (AAV-mPTEN-shRNA) and non-target control (AAV-scramble-shRNA) was evaluated for each timepoint.

Finally, the compound effect was investigated in five parameters that showed a consistent assay window, i.e., network-burst duration, spikes per network-burst, network-burst frequency, burst duration and spikes per burst. The effect of the compound was evaluated using a linear mixed effects model, comparing compound data with the PTEN KD condition.



PTEN reduction in mouse primary neurons leads to increased neuronal activity

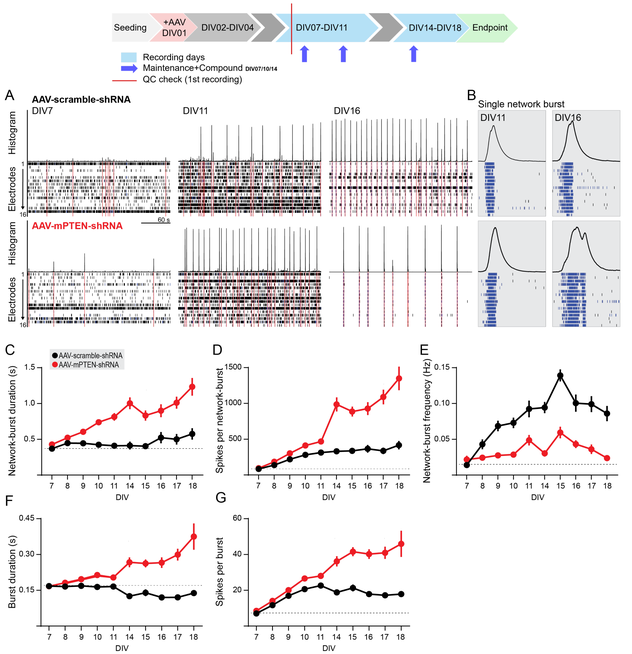
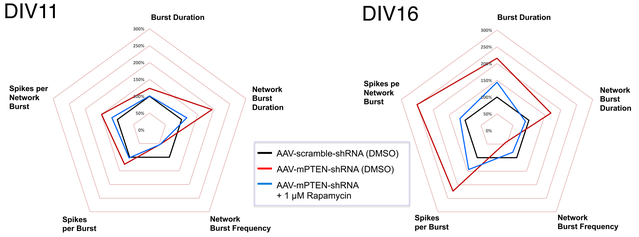


Figure 3. Mouse hippocampal neurons were plated into 48-well microelectrode array plates. Twenty-four hours after plating, neurons were transduced with an adeno-associated virus expressing a shRNA targeting PTEN. Electrophysiological phenotype was monitored with a period of 12 days with recordings on 10 days as shown. Multi-electrode array (MEA) analysis revealed five parameters were changed upon PTEN KD.

Conclusions

- PTEN KD induces a robust electrophysiological phenotype in primary neurons.
- Electrophysiological changes were detected even before the "neurite overgrowth".
- Treatment with 1 µM (but not 0.01 µM) Rapamycin largely prevented the development of the PTEN KD-induced electrophysiological phenotype.
- MEA assessment of neuronal network changes therefore presents a robust method to screen for drugs with the potential to treat PHTS neurological change.

The mTOR inhibitor Rapamycin prevents PTEN KD - induced neuronal activity in mouse neuronal model



Impact of rapamycin 0.01 and 1µM on neuronal activity – time course

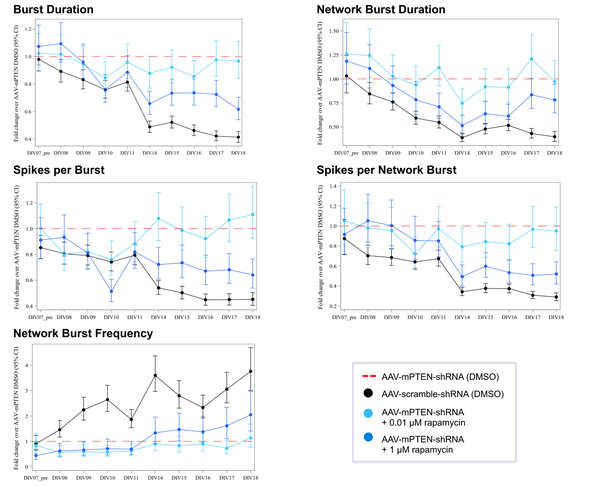


Figure 4. Neuronal activity of mouse hippocampal PTEN KD cells was monitored as described in Figure 3 and cultures were treated with with 0.01 µM or 1 µM rapamycin (with media change on DIV07, DIV10 and DIV14). Treatment with 1 µM Rapamycin resulted in significant reduction/increase of five activity related parameters towards AAV-scramble-shRNA control levels, indicating that high concentration rapamycin prevents the development of the PTEN KD phenotype. Upper panel shows results normalized to AAV-scramble-shRNA control, visualized in a spider web for 1 µM Rapamycin treatment at two individual days (DIV11 and DIV16). Lower panel shows the complete time course, with parameter values presented as fold change over AAV-mPTEN-shRNA. Confidence intervals (CI) that do not intersect y=1 represent statistically significant effects compared to the AAV-mPTEN-shRNA condition. Data are shown as mean ± 95% CI.

References

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