iPSC-Derived Neurons Harboring a Known Epilepsy Mutation Display Known and Novel Electrophysiological Phenotypes.

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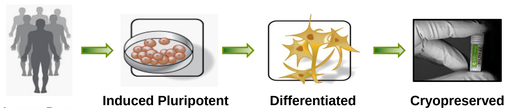
Abstract

Epilepsy is a disturbance in the electrical activity of the brain manifested via countless etiologies. 65 million individuals suffer from epilepsy and one-third of these individuals live with uncontrollable seizures because no known pharmacological treatment works for them. A portion of this population is accounted for by single-gene epilepsy disorders resulting from mutations within sodium, potassium or inhibitory channels. For example, the Slack gene (KCNT1) encodes a sodium-activated potassium channel that is very widely expressed in the brain. Mutations in this KCNT1 gene in humans presents with autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE), a disease marked by brief, but violent, seizures during sleep and devastating effects on intellectual function. Advances in personalized medicine is crucial for these types of diseases.

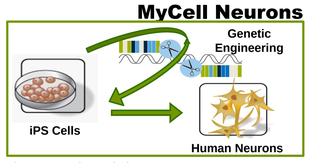
Central to this vision is induced pluripotent stem (iPS) cell technology, which provides a platform to expand our understanding of how single-gene mutations result in disease states. This approach illustrates and leverages the "disease-in-a-dish" iPSC-technology into phenotypic screening and drug development.

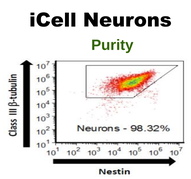
We have engineered and generated human cortical neurons harboring the *KCNT1* {P924L} single-gene mutations, as well as the isogenic wild-type control match. This ability provides unprecedented access to *in vitro* models of all-types of neurological disorders. Here we present functional data, via patch-clamp and multi-electrode array (MEA) electrophysiological techniques, illustrating the known 'gain-of-function' ionotropic cellular-level fingerprint, which has previously been linked to this mutation, along with newly-discovered neural-network level hyper-active phenotypes. We further show multiple examples that selective pharmacology can reverse these observed phenotypes. Collectively, our results illustrate how human iPS cells can be model disease states and be leveraged in the personal medicine space.

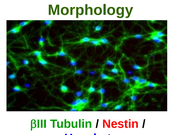
Human iPSC-derived Neuronal Cell Types

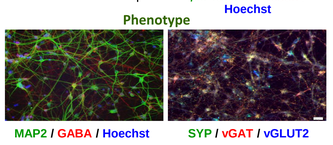


We utilize iPSC technology to reprogram adult cells (from either skin or blood) back to the "stem cell" state, then terminally differentiate these 'stem cells' into neurons (>95%) and finally cryo-preserve these neurons for immediate thaw and use. **iCell GABAneurons** are a population of predominately inhibitory neurons, but also contain some excitatory neurons. **Genetic Engineering** was utilized to introduce a single-gene mutation, KCNT1 {P924L}, into iCell GABANeurons, thus creating both epilepsy-harboring neurons as well as 'isogenic' control neurons. Here we investigate these two conditions to uncover appropriate and novel cellular alterations induced by this epilepsy mutation.

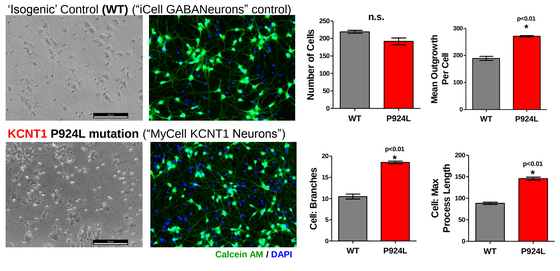






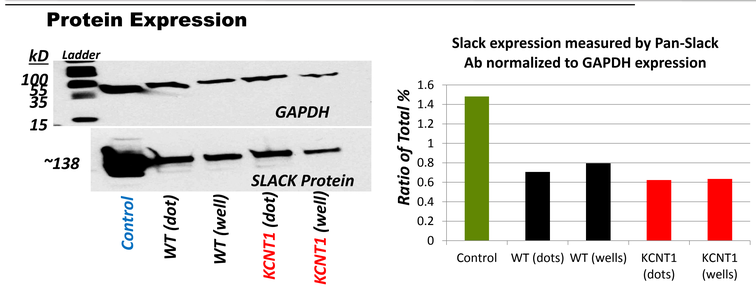


KCNT1 {P924L} Enhances Neurite Outgrowth



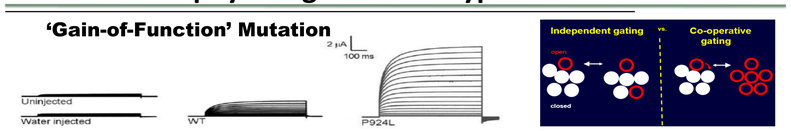
Isogenic control and KCNT1 {P924L}-harboring neurons were analyzed for neurite outgrowth properties by high content imaging (MetaXpress, Molecular Devices). At equivalent cell densities, KCNT1{P924L}-harboring neurons display increased outgrowth, branch number and maximum process length compared to isogenic control.

KCNT1 {P924L} 'Gain-of-Function'

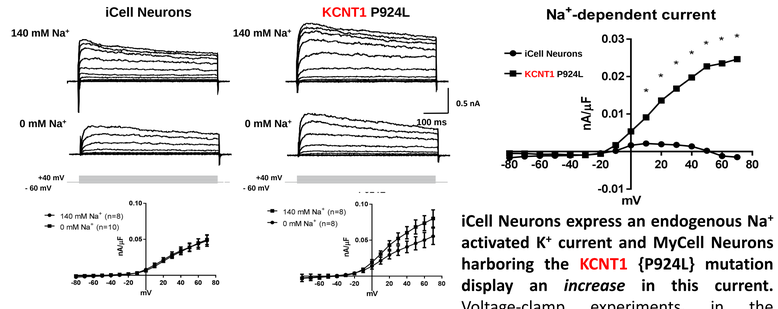


SLACK protein levels are not altered in mutant MyCell KCNT1(P924L) Neurons compared to control. Tissue samples were gathered from Day 9 cultures prepared from both iCell Neurons ('WT') and MyCell KCNT1{P924L} Neurons ('KCNT1') for MEA dotting ('dot') or regular cell culture ('well'). Evaluation via Western Blot of total SLACK protein and GAPDH was performed, along with the positive-'control' SLACK recombinant protein from Xenopus oocytes samples for verification. Total protein levels were normalized by GAPDH expression and samples were then ratio'd to determine if any conditions displayed different SLACK protein expression levels. No differences were observed.

Known Electrophysiological Phenotype



KCNT1{P924L} is a gain-of-function mutation. Expression of the Na^+ activated K^+ channel (KCNT1) P924L mutation in Xenopus oocytes shows an increase in current amplitude compared to wild-type KCNT1 channels. **Milligan et al. Ann. Neurol. (2014) 75: 581-590.** The 'gain-of-function' phenomenon is known to be produced by co-operative gating of active, mutant KCNT1 channels.

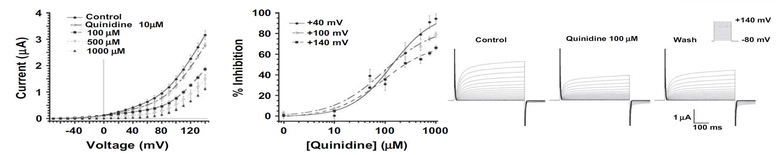


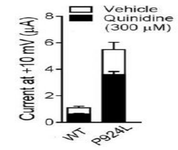
iCell Neurons express an endogenous Na^+ activated K^+ current and MyCell Neurons harboring the KCNT1 {P924L} mutation display an *increase* in this current. Voltage-clamp experiments, in the presence or absence of Na^+, display outward membrane currents generated from voltage steps (-60 to +40 mV). At more depolarizing voltages, MyCell KCNT1 Neurons display an increased amount of this outward current compared to iCell Neuron control neurons. These data parallel Xenopus oocyte findings.

Conclusions

- Human iPSC-derived neurons display *KNOWN* electrophysiological behaviors
  - *'Slack' channel Na+ activated K+ outward membrane currents*
- MyCell KCNT1{P924L} Neurons display *KNOWN* outward current behaviors
  - *'Gain-of-function' increases in 'Slack' channel currents & co-operative gating*
- MyCell KCNT1{P924L} Neurons display *NOVEL* MEA-measured bursting
  - *'Poisson' bursting behaviors are 'hyper-active'*
- Quinidine ameliorates *NOVEL* 'hyper-active' KCNT1 bursting behaviors
  - *'Poisson' bursting is abolished and network-level bursting is dampened by Quinidine*

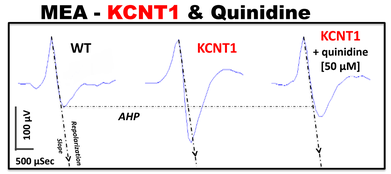
Quinidine Pharmacology

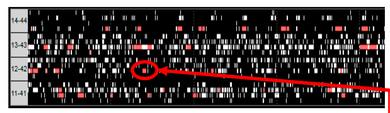




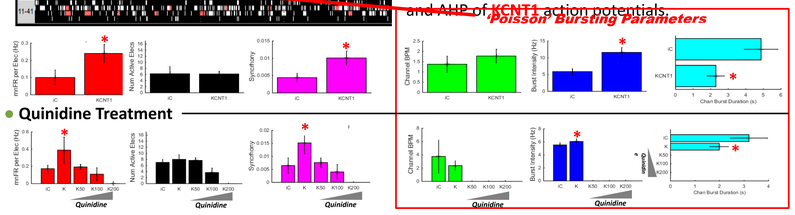
Quinidine blocks *Slack* currents in a concentration-dependent manner in Xenopus oocytes, with calculated EC50 values of 138.2, 94.4 and 91.6 μM at +40, +100 and +140 mV, respectively (above). Figure adapted from **Yang et al. Neuropharm. (2006) 51: 896-906.** A second study further showed that Slack channels harboring the P924L mutation specifically were also sensitive to inhibition with Quinidine, suggesting neurons with this mutation can benefit from Quinidine treatment (left). **Milligan et al. Ann. Neurol. (2014) 75: 581-590.**

Novel Electrophysiological Phenotype

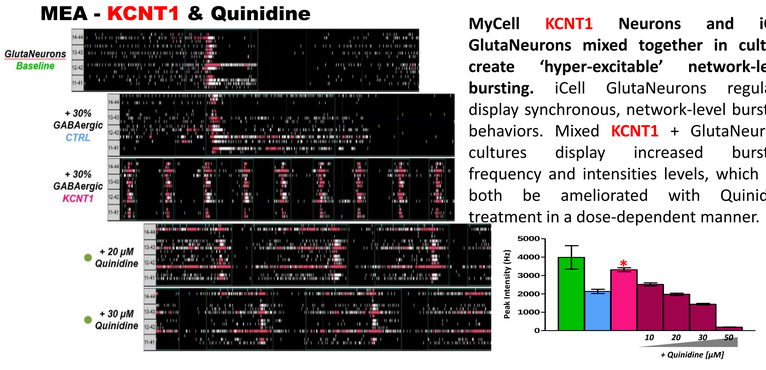




High-resistance, single-unit multi-electrode array (MEA) recordings allow individual waveforms to be captured, parsed (PCA), and compared across different conditions, before and after drug treatment. Untreated KCNT1 neuronal action potential waveforms (**middle trace**) display a quickened repolarization slope and a larger fast after-hyperpolarization (fAHP) compared to WT (**left trace**) (average of ≥80 action potentials per trace). Treatment of 50 μM quinidine onto KCNT1 neurons (**right trace**) dampens both the repolarization slope and AHP of KCNT1 action potentials.



MyCell KCNT1 Neurons display more intense and shorter 'Poisson' bursting behaviors compared to WT. Neuronal cell cultures of both WT iCell Neurons (iC) and MyCell KCNT1 Neurons (KCNT1) (Day 10) display spontaneous activity (raster plots) and bursts (colored tick-marks) that can be measured via MEA. Quinidine treatment of KCNT1 cultures ameliorates the increased mean firing rate (red bars) and synchrony (purple bars) observed in these cultures. Furthermore, quinidine eliminated 'Poisson' bursting in KCNT1 neurons.



MyCell KCNT1 Neurons and iCell GlutaNeurons mixed together in culture create 'hyper-excitable' network-level bursting. iCell GlutaNeurons regularly display synchronous, network-level bursting behaviors. Mixed KCNT1 + GlutaNeurons cultures display increased bursting frequency and intensities levels, which can both be ameliorated with Quinidine treatment in a dose-dependent manner.