A FRET-BASED HIGH-THROUGHPUT DRUG SCREEN TARGETING DYSREGULATED INTRACELLULAR CALCIUM SIGNALING IN ALZHEIMER’S DISEASE

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Currently most of the drug development for Alzheimer’s disease (AD) target very late events in the disease progression, e.g. removal of Aβ pathology or reducing tangle formation. Alternatively, endoplasmic reticulum (ER) calcium dyshomeostasis can be used as a target for drug development. Familial Alzheimer’s disease (FAD) mutations in presenilins (PS1 and PS2) disrupt ER calcium signaling in an early event during the AD pathogenesis. FAD presenilin mutations enhance the inositol 1,4,5-trisphosphate (IP3) receptor activity which presents a potential novel target for AD therapy.

To perform a high-throughput drug screening, we established a fully automated FRET-based calcium imaging assay at single-cell-level on the Opera platform (PerkinElmer). We generated HEK293 cells co-expressing a disease causing mutated form of presenilin (FAD-PS1) and Yellow Cameleon 3.6 (YC3.6), a FRET-based calcium sensor. Agonist-induced IP3 production by carbachol (CCh) results in liberation of calcium from the ER. Most FAD-PS1 mutants show potentiated CCh calcium release compared to the controls.

Using the described FAD-PS1/YC3.6 HEK293 cells, we have conducted a high-throughput screen with a library of 20,000 small molecules. We identified hits which attenuate the CCh calcium release of FAD-PS1 cells in a dose dependent manner, some even to the level of wildtype PS1 cells. In order to present the lead structures, IP3 uncaging experiments are yet to be performed and structure-activity relationships to be determined.

Here we performed a high-throughput calcium-imaging-based drug screening and identified new potential therapeutic agents that target one of the upstream events in AD progression before the pathological hallmarks of AD manifest.