SCREENING UNIT FMP

http://www.leibniz-fmp.de/core-facilities/-screening-unit/

GERMANY

high-capacity site

BERLIN

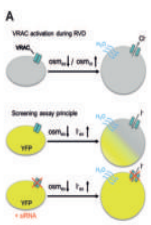
an official partner site of eu openscreen

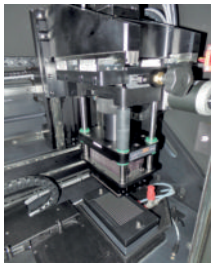
THE PEOPLE

Dr. Jens Peter von Kries (Head)
Dr. Katina Lazarow (Functional Genomics)
Dr. Silke Radetzki (High Content Screening)
Dr. Martin Neuenschwander (Data analysis, lab automation)
M. Sc. Marc Wippich (Image data analysis, IT)
Carola Seyffarth (Biochemical screening)
Sabrina Kleissle (Cell based screening)
Peggy Treffkorn (Cell based screening)
Andreas Oder (Biacore & biochemical screening)

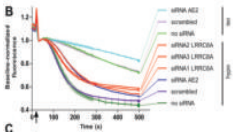
THE PROJECTS

Reference Project 1





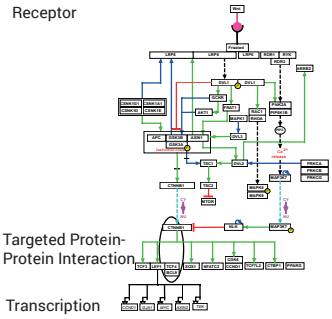
to be able to track the fast fluoresence changes immediately after hypotonic buffer addition, a real time fluorescence imager equipped with a 384-channel pipetting head was used (FLIPR TETRA)

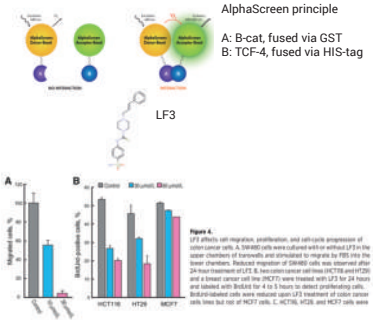


Suppression of LRRC8A expression led to lower activity of VRAC, visible as delayed fluorescence decrease compared to scrambled/no siRNA controls

Regulation of cell volume is critical for many cellular functions, yet the molecular identity of the key player VRAC (volume-regulated anion channel) remained unknown despite decades of efforts. VRAC is nearly inactive under resting conditions, but opens upon hypotonic swelling. Activity was assayed by using HEK cells constitutively expressing YFP, upon hypotonic swelling induced by addition of a iodine-containing low salt buffer, iodine influx could be observed indirectly by YFP fluorescence quenching. By transfecting cells with a human RNAi library that used three separate RNAi per gene, one RNAi probe succesfully detected the LRRC8A genes as being the responsible entity for VRAC activity.

Reference Project 2





Wnt/B-catenin signalling contributes to high cell motility, high cell proliferation, and cell-cycle progression in colon cancer cells. Activating mutations of Wnt signalling are often found in downstream components of the pathway. Using AlphaScreen technology, a small molecule library (ChemBioNet collection with a diversity set of 16'671 compounds) was searched for molecules that disrupt the B-catening/TCF-4 protein interaction, leading to the successful identification of LF3. LF3 is able to specifically reduce cell motility and proliferation in colon cancer cell lines, while not affecting other cancer cell lines.

THE HARDWARE

- 3 Tecan Freedom Evo and 1 Beckman FxP liquid handling workstation equipped with fixed tip and disposable tip 384-channel pipetting heads and intgrated cell incubators.
- Arrayscan VI high-content fluorescence microscopes
  EZReader II capillary electrophoresis system for kinases/phosphatases
  Miltenyi MACSQuant flow cytometer for epitopes and genetic reporters
  FLIPR TETRA for ion-channels
  Plate readers equipped for HTRF, luminescence, and fluorescence based readouts
- The small-molecule library consitsts of a diversity set (33'088), academic compounds collected from multiple groups across Germany (6'424), and a reference set with FDA approved drugs, drug candidates and LOPAC (3'168), and is managed in a fully automated tube store at -20°C.

THE SOFTWARE

- Konstanz Information Miner (KNIME) for data analysis and reporting
- R statistics framework for IC50 plot generation (using automated outlier detection after Motulsky et al.)
- MySQL for frequent hitter database
- DACS, database of accessible chemical substances, in-house software for compound search and library design
  http://www.leibniz-fmp.de/de/ssfa0/database-dacs.html

- The functional genomics libraries consist of a human and mouse RNAi library (with subsets available for kinases) and is currently expanded to CRISPR/CAS9 libraries.

THE OUTPUT

1. *Du J, et al. (2017) Pharmacological restoration and therapeutic targeting of the B-cell phenotype in classical Hodgkin lymphoma. Blood 129, 71–81*
2. *Wetzel C, et al. (2017) Small-molecule inhibition of STOML3 oligomerization reverses pathological mechanical hypersensitivity. Nat. Neurosci 20, 209-218*
3. *Chenge JT, et al. (2016) Structural characterization and ligand/inhibitor identification provide functional insights into the Mycobacterium Tuberculosis Cytochrome P450 CYP126A1. J. Biol. Chem. 292, 1310-1329*
4. *Fang L, et al. (2016) A Small-molecule antagonist of the β-Catenin/TCF4 interaction blocks the self-renewal of cancer stem cells and suppresses tumorigenesis. Cancer Res. 76, 891–901.*
5. *Khatri Y, Ret al. (2016) Substrate hunting for the myxobacterial CYP260A1 revealed new 1α-hydroxylated products from C-19 steroids. Chembiochem 17, 90–101.*

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THE FUTURE

- upgrade to acoustic dispensing technology
- installation of BSL2 HTS lab

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