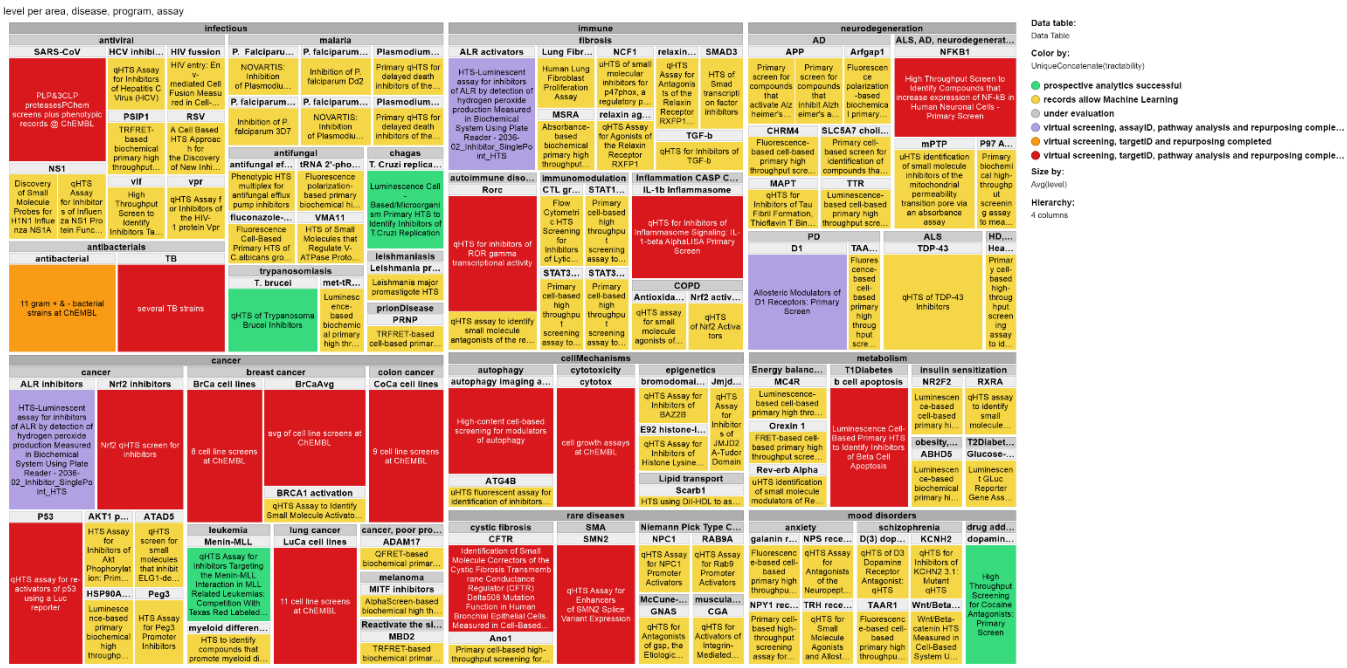
DrTarget Portfolio

Catalogue of projects suitable for machine Learning

More than 100 projects recorded in ChEMBL and PubChem DBs evaluated to determine their tractability for predictive analytics and classified in three levels. .

- Level 1: The project contains enough records to apply machine learning methodologies.
- Level 2: Prospective analytics successful.
- Level 3: Virtual screening, target ID, pathways analysis ad drug repurposing completed and compared to existing data.



PubChem Information Sources

1. PubChem

   Data deposited in or computed by PubChem
   https://pubchem.ncbi.nlm.nih.gov

2. BioAssay Research Database (BARD)

   1530
   https://bard.nih.gov/BARD/experiment/show/1530

Projects Summary Table

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cancer	cancer	Peg3	1
cancer	cancer	Nrf2 inhibitors	1
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cancer	leukemia	myeloid differentiation	1
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cellMechanisms	epigenetics	E92 histone-lys N-methyltransferase 2	1
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immune	COPD	Antioxidant Response Element	1
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immune	fibrosis	relaxin agonists	1
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immune	fibrosis	MSRA	1
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immune	fibrosis	SMAD3	1
immune	fibrosis	TGF-b	1
immune	immunomodulation	CTL granule exocytosis	1
immune	immunomodulation	STAT1 activation	1
immune	immunomodulation	STAT3 activation	1

immune	immunomodulation	STAT3 inhibition	1
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infectious	antifungal	antifungal efflux pumps inhibitors	1
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infectious	antifungal	tRNA 2'-phosphotransferase	1
infectious	antiviral	SARS-CoV	3
infectious	antiviral	HCV inhibitors	1
infectious	antiviral	PSIP1	1
infectious	antiviral	vpr	1
infectious	antiviral	vif	1
infectious	antiviral	HIV fussion	1
infectious	antiviral	RSV	1
infectious	antiviral	NS1	1
infectious	antiviral	NS1	1
infectious	antiviral	hCMV	0
infectious	chagas	T. Cruzi replication	2
infectious	leishmaniasis	Leishmania promastigote	1
infectious	malaria	Plasmodium delayed death (apicoplast)	1
infectious	malaria	Plasmodium cell death	1
infectious	malaria	P. falciparum 3d7	1
infectious	malaria	P. falciparum Dd2	1
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infectious	trypanosomiasis	met-tRNA synt	1
metabolism	Energy balance, obesity	Orexin 1	1
metabolism	Energy balance, obesity	MC4R	1
metabolism	Energy balance, obesity	Rev-erb Alpha	1
metabolism	insulin sensitization	NR2F2	1
metabolism	insulin sensitization	RXRA	1
metabolism	obesity, diabetes, and cardiovascular disease	ABHD5	1
metabolism	T1Diabetes	β cell apoptosis	3
metabolism	T2Diabetes	Glucose-dependent insulin secretion	1
mood disorders	anxiety	TRH receptor enhancers	1
mood disorders	anxiety	NPS receptor antagonists	1
mood disorders	anxiety	NPY1 receptor antagonists	1
mood disorders	anxiety	galanin receptor antagonists	1

mood disorders	drug addiction	dopamine transporter	2
mood disorders	schizophrenia	TAAR1	1
mood disorders	schizophrenia	D(3) dopamine receptor	1
mood disorders	schizophrenia	Wnt/Beta-catenin pathway	1
mood disorders	schizophrenia	KCNH2	1
neurodegeneration	AD	IDE	1
neurodegeneration	AD	APP	1
neurodegeneration	AD	APP	1
neurodegeneration	AD	CHRM4	1
neurodegeneration	AD	TTR	1
neurodegeneration	AD	Arfgap1	1
neurodegeneration	AD	MAPT	1
neurodegeneration	AD	SLC5A7 choline transporter	1
neurodegeneration	ALS	TDP-43	1
neurodegeneration	ALS, AD, neurodegeneration	NFKB1	3
neurodegeneration	ALS, AD, neurodegeneration	mPTP	1
neurodegeneration	ALS, AD, neurodegeneration	P97 ATPase	1
neurodegeneration	HD, AD, PD, ALS, neurodegeneration	Heat shock 70kDa protein 1A	1
neurodegeneration	PD	TAAR1	1
neurodegeneration	PD	D1	3
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rare diseases	cystic fibrosis	Ano1	1
rare diseases	McCune-Albright syndrome (MAS)	GNAS	1
rare diseases	muscularDistrophy	CGA	1
rare diseases	Niemann Pick Type C (NPC)	NPC1	1
rare diseases	Niemann Pick Type C (NPC)	RAB9A	1
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Projects description and contact information.

Extracted from depositors' summary in PubChem.

1. Cancer

a. Breast Cancer

i. Phenotypic screens: 8 BrCa cell line screens at ChEMBL

ii. BRCA1

Description

Functionally, BRCA1 has been implicated in a wide array of cellular activities, including DNA damage repair, cell-cycle checkpoint control, growth inhibition, apoptosis, transcriptional regulation, chromatin remodeling, protein ubiquitylation, and mammary stem cell self-renewal and differentiation. Reduction in BRCA1 levels is associated with an increase in tumor growth and resistance to anti-estrogenic agents, such as tamoxifen, while an increase in BRCA1 expression leads to cell growth arrest and apoptosis. Furthermore, in vitro and in vivo studies have revealed that loss of BRCA1 expression in mammary epithelial cells leads to failure of terminal luminal epithelial cell differentiation, exaggerated expression of basal/myoepithelial antigens, and enrichment of cells with expression of the putative stem/progenitor cell marker ALDH1A1. The frequent loss of BRCA1 expression in invasive breast cancers in the absence of somatic mutation has been attributed to numerous mechanisms, including hypermethylation of the BRCA1 promoter, exaggerated ubiquitylation of BRCA1 protein, and improper intracellular localization. Transcriptional profiling of large cohorts of BRCA1-mutated breast cancers has revealed that these tumors, almost without exception, cluster in the basal-like subtype. Like breast tumors which arise in BRCA1 mutation carriers, sporadic cancers which demonstrate somatic silencing or dysfunction of BRCA1 are also of the basal-like phenotype. Basal-like breast cancers (BLBCs) are associated with an aggressive clinical course, resistance to chemotherapy, and typically lack expression of estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2). BLBCs account for 10-17% of all breast cancers, have few therapeutic options, and are considered the biggest unmet need in the treatment of breast cancer. Conversely, tumors which maintain expression of functional BRCA1 are almost uniformly luminal type cancers, and are accordingly associated with more indolent clinical courses, responsiveness to endocrine therapies, and improved survival.

As such, it is possible that an increase in BRCA1 expression would enable cellular differentiation and restore tumor suppressor function, resulting in delayed tumor growth and less aggressive, more treatable breast cancers. The long term goal of this proposal is to develop a novel prevention or therapeutic agent for breast cancer patients. To accomplish this goal, the overall objective of this proposal is to access the resources provided by the NIH Molecular Libraries Roadmap Initiative and the Molecular Libraries Probe Production Centers Network (MLPCN) to discover and develop new chemical activator probes that will increase BRCA1 expression and function.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
Grant: MH093215
PI Name: Lisa Harlan-Williams, University of Kansas Medical Center

b. Cancer

i. P53

Description

The p53 tumor suppressor plays a key role in suppression of tumorigenesis and in human carcinogenesis. It is frequently found inactivated in human cancers, mainly by point mutation. These point mutations are found in a wide variety of human cancers and a majority of them affect the DNA binding domain of the protein. The point mutations either alternative p53 conformation or abolish DNA contact. Additionally, cancer cells with p53 mutations are more resistant to chemo-radiation therapies.

In order to find activators to reactivate this important protein, a cell-based screen for p53-dependent luciferase-reporter expression was developed. A p53-null H1299 lung cancer cell line was stably co-transfected with a temperature sensitive p53 mutant, p53-A138V, and a p53-responsive BP100-luciferase reporter. This cell model has previously been characterized in a gene chip profiling experiment for p53 activation. By lowering the culture temperature from 37 deg C (mutant p53 conformation) to 32 deg C (w.t. p53 conformation), a 30-60 fold increase in activation of luciferase activity is observed. At assay temperate, 37 deg C, basal reporter expression is very low and after the addition of detection reagent, a luminescent read-out is carried out.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production Centers Network [MLPCN]
MLPCN Grant: DA031088-01A1
Assay Provider (PI): Yi Sung, University of Michigan at Ann Arbor

ii. HSP90AA1

Description:

Hsp90 is a ubiquitously conserved molecular chaperone that assists the folding of client substrates involved in a variety of human disorders characterized by dysregulated cell proliferation (ie, cancer and viral infections), accumulation of protein aggregates (ie, neurodegenerative diseases) and stress-induced apoptotic cell death (ie, multiple sclerosis). Its diverse biology stems in part from interactions with numerous kinases and transcription factors (3). In addition, Hsp90 facilitates protein folding via interactions with a set of ancillary proteins (co-chaperones) and the binding and hydrolysis of ATP.

A role for Hsp90 in tumorigenesis is supported by the fact that it is the only chaperone with differential activities in cancer and normal cells. Hsp90 exists primarily as an uncomplexed homodimer in normal cells, and predominantly in complexes with clients and co-chaperones in malignant cells. In malignant cells, Hsp90 is over-expressed and is required to fold and maintain the activity of both native and mutated signal transduction proteins responsible for uncontrolled proliferation. Hsp90 in these multi-protein complexes has a much higher affinity for ATP, which is required for the Hsp90-mediated protein folding process, compared to dimeric Hsp90 found in normal cells. Because of the essential role of Hsp90 in multiple signaling cascades and protein aggregation, Hsp90 inhibitors are attractive

potential therapies for cancers and neurodegenerative disorders. Although Hsp90 inhibitors exist and are currently under study in clinical trials, problems such as cytotoxicity and poor solubility demand that novel compounds targeting Hsp90 be identified.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: Robert Matts, Oklahoma State University
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 X01 MH083240-01
Grant Proposal PI: Robert Matts, Oklahoma State University
External Assay ID: HSP90_INH_LUMI_1536_%INH

iii. ATAD5

Description

Cancer cells divide rapidly compared to normal cells in the body. During cell division, cancer cells need to duplicate their genome by DNA replication. The failure of genome duplication leads to cancer cells' death. Therefore, inhibitors of DNA replication could specifically kill cancer cells. Based on this concept, many chemotherapeutic agents were developed and have been used to treat cancer patients. However, there are a limited number of agents available in the clinical setting, and some of those that do exist have low efficacy and severe side effects. Therefore, if we can find the compounds that effectively block DNA replication either by directly damaging DNA or by inhibiting other cellular mechanisms, the options for cancer treatment will be broadened. With this in mind, we developed a quantitative high-throughput ELG1-luciferase reporter gene assay. Small molecules that specifically block ELG1 function in response to DNA damage can be identified by monitoring the suppression of luciferase activity. Since ELG1 defines a pathway for repairing DNA damage, agents blocking ELG1 function can be used to sensitize cancer cells to chemotherapeutic agents whose mechanism of action involves the induction of DNA damage.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
MLPCN Grant: 1 R03 MH092164-01
Assay Provider: Kyungjae Myung, NHGRI, NIH

iv. Peg3

Description

Cancer is a progressive disease culminating in the acquisition of metastatic potential by a subset of evolving tumor cells. Although extensively investigated, the precise molecular events underlying tumor development and cancer progression remain unclear. Progression elevated gene-3 (PEG-3), originally identified in rodents, displays elevated expression as cancers become more aggressive. The minimal promoter region controlling PEG-3 expression, PEG-Prom, has been isolated and shown to display elevated expression in a wide range of both human and rodent tumors, with minimal expression in normal cells. The PEG-Prom is transcriptionally activated following transformation by diverse acting oncogenes or as a consequence of unidentified genetic factors mediating cellular transformation. We have determined the mechanism for this selectivity and it involves transcription (gene regulatory) factors, AP-1 and PEA-3, which are expressed at elevated levels in virtually all rodent and human cancers. Moreover, in cases of cancer reversion or blocking expression of specific transforming oncogenes, PEG-Prom activity is decreased. In these contexts, the PEG-Prom represents a valuable predictive tool and readout to identify molecules with potential

antitumor activity, without a priori identification of the genetic changes causative of the transformed/tumorigenic phenotype. The primary goal of this screen is to identify chemical inhibitors of the PEG-Promoter generated from high-throughput screening methods. These small molecule chemical probes might have potential anticancer properties and could provide a foundation for the development of novel antitumor drugs.

Data Source: Sanford-Burnham Center for Chemical Genomics (SBCCG)
Source Affiliation: Sanford-Burnham Medical Research Institute (SBIMR, San Diego, CA)
Network: NIH Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Number: 2R01 GM068385-06
Assay Provider: Paul B. Fisher, M.Ph., Ph.D., Virginia Commonwealth University

v. AKT1 phosphorylation

Description

The serine/threonine protein kinase Akt (protein kinase B) regulates various cellular processes, including cell survival, growth, proliferation, migration and differentiation. It has been well established that Akt is activated through two essential steps involving membrane translocation and phosphorylation. Cytosolic Akt is recruited to the plasma membrane via the interaction between the PH domain and membrane PIP3 produced by phosphoinositide-3 kinase (PI3K) upon growth factor receptor stimulation. The membrane-Akt interaction results in conformational changes of Akt, enabling its activation through phosphorylation at T308 in the kinase domain and at S473 in the C-terminal hydrophobic motif by PDK1 and mTOR-rictor complex (mTORC2), respectively. Although T308 phosphorylation by PDK1 is indispensible for Akt activation, T308 phosphorylation is significantly boosted by prior phosphorylation of S473. Consistently, S473 phosphorylation has been shown to enhance Akt kinase activity by 4-5 fold. Conversely, knock-down of mTORC2 significantly reduces T308 phosphorylation by PDK1. It has been suggested that the non-phosphorylated C-terminal hydrophobic motif is involved in sustaining the inactive conformer of Akt. These findings indicate that S473 phosphorylation, as an important modulator of Akt activation, may be a valuable drug target for cell growth, survival, proliferation and differentiation. These inhibitors also may have significant therapeutic potential with fewer side effects, especially for the conditions involving hyperactive Akt signaling, such as cancer and Alzheimer's disease.

To this end, a miniaturized assay was developed to be screened against the Molecular Libraries Small Molecule Repository (MLSMR).

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH093214
Assay Submitter (PI): Hee-Yong Kim, National Institute on Alcohol Abuse and Alcoholism

vi. NFE2L2 inhibitors

Description

Nrf2 is a transcription factor that maintains cellular redox homoestasis and protects cells from xenobiotics. Nrf2 binds to the antioxidant response element (ARE) to induce gene expression of a broad spectrum of genes that encode for antioxidants. Hence this Nrf2 pathway provides a first line of defense against stress caused by exposure to radiation, electrophiles, and xenobiotics. In many cancers it has been found that tumor cells have manipulate the Nrf2 pathway for their survival against cytotoxic chemotherapeutics and radiotherapeutic agents. Finding a small molecule that act as an inhibitor of Nrf2 function

would represent a novel therapeutic target that could lead to improvement in survival of patients undergoing chemo- and/or radio- therapy.
This is the Nrf2 activity measured in the luminescent read.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
MLPCN Grant: R03-MH092170-01
Assay Provider: Shyam Biswal, Johns Hopkins University

vii. ALR inhibitors

Description

The goal of this project is to develop small molecule modulators that affect the mitochondrial sulfhydral oxidase pathways by using purified components, specifically ALR, in an in vitro assay.
Defects in mitochondrial assembly impact a wide range of diseases from degenerative muscle and neural diseases to cancer. The mitochondrion is not only important for the production of energy but plays an important role in other aspects such as intermediary metabolism and signaling. The mitochondrion contains an inner membrane and outer membrane that separate the matrix from the intermembrane space. The intermembrane space is an important compartment because it houses molecules such as cytochrome c and apoptosis inducing factor (AIF) that are released during apoptosis. Recently, the intermembrane space has been implicated in diseases such as Parkinson's disease by the presence of the kinase PINK1. In addition, we continue to make new discoveries about the function of this compartment. Specifically, we have identified a new import pathway in the intermembrane space that is redox regulated, indicating that oxidation-reduction reactions are unexpectedly housed in this compartment. Key components include the sulfhydryl oxidase Erv1 and cytochrome c, which form a complex in the intermembrane space. A recent study has demonstrated a role of ALR, the human Erv1 homolog, in Autosomal-Recessive Myopathy. Both Erv1 and ALR belong to a class of FAD dependent sulfhydryl oxidases that have essential roles in the mitochondrion.
Screen for inhibitors of purified, recombinant ALR to oxidize the generic substrate DTT. Oxidation of DTT by ALR produces hydrogen peroxide, which was detected using horseradish peroxidase (HRP) and luminol. A solution of ALR and HRP was added to plates containing compounds, followed by the addition of DTT. The plates were incubated for 2 hours prior to the addition of the luminol solution. After a minute incubation, plates were read to detect the flash luminescence signal.

Primary Collaborators: Carla Koehler,UCLA,Los Angeles,CA,koehlerc@chem.ucla.edu,310-794-4834. Deepa Dabir,UCLA,Los Angeles,CA,deepad@chem.ucla.edu
Samuel Hasson,UCLA,Los Angeles,CA,hassons@gmail.com

c. Cancer, poor prognosis EGFR overexpressing tumours.

i. ADAM17

Description

Approximately 20-30% of breast cancer patients have tumors that over-express human epidermal growth factor receptor (HER2), which confers an aggressive tumor phenotype and poor prognosis. A Disintegrin and Metalloprotease (ADAM) proteases are responsible for amplification of HER2 signaling due to either cleavage of its extracellular domain or release

of HER2 ligands, which leads to proliferation and inhibition of apoptosis. ADAM proteases implicated in amplification of HER2 signaling are ADAM10 and 17; therefore, inhibition of these proteases represents a viable approach to the abrogation of HER2 signaling in breast cancer. The specific aims of this proposal, therefore, will focus on (1) screening of the MLPCN library for inhibitors that interact with exosites of ADAM10 and 17, and (2) medicinal chemistry optimization of initial leads in order to develop molecular probes of ADAM10 and 17. Our laboratory is uniquely positioned to achieve these goals due to expertise in development of exosite-binding inhibitors and probes, HTS, and biochemistry of proteases. We will also collaborate with experts in the fields of peptide synthesis, HTS, and medicinal chemistry. The successful completion of the Aims of this proposal will lead to a discovery of novel, potent, and selective small molecule probes for ADAM10 and 17. Using these selective molecular probes alone or in combination with other tools, such as siRNA, antibodies, and other small molecule inhibitors, the researchers will be able to study contributions not only of individual members of the ADAM protease family, but also the interplay of ADAM protease-controlled pathways with other pathways implicated in the progression of breast cancer.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Center Affiliation: Torrey Pines Institute for Molecular Sciences (TPIMS)
Assay Provider: Dmitriy Minond, Torrey Pines Institute for Molecular Sciences (TPIMS)
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R03 DA033985-01
Grant Proposal PI: Dmitriy Minond, Torrey Pines Institute for Molecular Sciences (TPIMS)
External Assay ID: ADAM17_INH_QFRET_1536_1X%INH PRUN

d. Leukemia

i. Menin-MLL

Description

Translocations of the MLL (Mixed Lineage Leukemia) gene are frequently found in human leukemias affecting both children and adults. Fusion of MLL to one of more than 60 genes results in generation of oncogenic proteins upregulating Hox genes, which are vital to blood cell development. Patients harboring fusion of the MLL gene suffer from aggressive leukemias and respond poorly to available therapies. All of the oncogenic fusion proteins have a preserved N-terminal fragment of MLL that has been identified to interact with menin. It has been recently discovered that association with menin is critical to the leukemogenic activity of the MLL fusion oncoproteins. Selective targeting of the menin-MLL interaction could provide an attractive therapeutic approach to develop novel drugs for MLL-related leukemias. Small molecules blocking the menin-MLL interaction should reverse the oncogenic potential of MLL fusion proteins.

We have developed and optimized a fluorescence polarization-based assay for the identification of Menin-MLL inhibitors. The assay was run employing two different MLL-derived peptides. The present assay uses a 12 amino acid peptide labeled with fluorescein at its N-terminus. This peptide consists of the menin high affinity binding motif from MLL and is potently bound by menin. Another peptide with a mutated sequence, labeled with Texas Red, was used in parallel as a less stringent screen for inhibitors.

qHTS Assay for Inhibitors Targeting the Menin-MLL Interaction in MLL Related Leukemias

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH084875-01A1
Assay Submitter (PI): Jolanta Grembecka, Tomasz Cierpicki, University of Virginia

ii. myeloid differentiation

Description

The potential for successful differentiation therapy in acute leukemia was realized with the clinical development of all-trans retinoic acid (ATRA) and arsenic. ATRA and arsenic overcome the differentiation arrest imposed by the retinoic acid receptor alpha (RARa) fusion oncoprotein and treated leukemic promyelocytes terminally differentiate to mature neutrophils. These small molecules are remarkably well-tolerated by patients in comparison to traditional cytotoxic chemotherapy. Furthermore, incorporating ATRA into treatment regimens single-handedly improved the overall survival of patients with acute promyelocytic leukemia (APL) from 20% to 75%. Unfortunately, differentiation therapy does not exist for the much larger fraction of non-APL acute myeloid leukemias where the standard of care results in an overall survival rate of only 25%.

The mammalian homeobox transcription factors contribute to lineage-specific hematopoietic differentiation, and their expression is tightly regulated during normal hematopoiesis. Though critical to early hematopoiesis, the expression of the HoxA cluster of genes is normally downregulated as cells mature. The persistent, and inappropriate, expression of members of the HoxA cluster of genes has been described in the majority of acute myeloid leukemias. More specifically, HoxA9 has an important role in normal hematopoiesis and leukemogenesis. HoxA9 is directly involved in human leukemias as one partner of the fusion protein NUP98- HoxA9. In analyses of human AML, the level of HoxA9 expression has been correlated with poor prognostic karyotype and inversely correlated with survival. Furthermore, in patients with CML, a relatively higher level of HoxA9 expression was associated with transition from chronic phase to accelerated and blast phase. More recently it has been shown that HoxA9 is critical to the small subset of lymphoid and myeloid leukemias that express fusion oncoproteins involving the mixed lineage leukemia (MLL) gene. Leukemias harboring MLL-rearrangements are a particularly poor prognosis subgroup of AML and depend on HoxA9 for proliferation and survival. Overall, this suggests that HoxA9 dysregulation - via fusion with NUP98 or via inappropriate maintenance of HoxA9 expression - is a common pathway in the differentiation arrest in myeloid leukemia. This is an exciting possibility, as it suggests that by specifically targeting this pathway, one might be able to overcome differentiation arrest.

The identification of differentiation therapy has been hindered by the lack of a good model system of differentiation arrest in acute myeloid leukemia. Primary leukemic cells are difficult to isolate and culture, and their availability is limited. Leukemia cell lines (e.g. HL60, NB4, 32D) are readily available though their underlying mechanism of differentiation arrest is not known. Furthermore, these cell lines are only capable of incomplete differentiation and they differentiate in response to non-physiologic stimuli (e.g. DMSO, PMA). Finally, differentiation is cumbersome to assay in a high-throughput fashion, and previous studies have focused on complex screens using qPCR to monitor small changes in gene expression. These problems have made it very difficult to adopt a system for the purpose of high-throughput screening to identify compounds which promote differentiation.

We have devised a novel cell-based assay with advantages over existing systems. The assay (1) provides an unlimited supply of cells, (2) the cells are derived from primary marrow, (3)

the differentiation arrest is imposed by a single and clinically relevant oncoprotein, (4) the cells have a built-in marker of differentiation, and (5) when the oncoprotein is inactivated or inhibited, the cells are capable of recapitulating full and normal myeloid differentiation.

Assay Support: 1 R03 DA032471-01
Project Title: Discovering small molecules that overcome differentiation arrest in acute myeloid leukemia
PI: David Sykes, PhD
Screening Center PI: Larry Sklar, PhD / UNMCMD
Screening Center Manager: Kristine Gouveia
Screening Lead: Mark Haynes, PhD
Assay Implementation: Mark Haynes, Stephanie Chavez
Chemistry Center PI: Stuart Schreiber, PhD / BIPDeC
Chemistry Center Manager: Patti Aha
Chemistry Lead: Tim Lewis

e. Lung Cancer

i. Phenotypic screens: 8 LuCa cell line screens at ChEMBL

f. Melanoma

i. MITF inhibitors

Description

Microphthalmia transcription factor (MITF) is a master transcription factor expressed in melanocytes, essential for melanocyte survival, differentiation, and pigment formation, and is a key oncogenic factor in melanoma initiation, migration, and treatment resistance. Although identified as an important therapeutic target for melanoma, clinical inhibitors directly targeting the MITF protein are not available. Based on the functional state of MITF, we have designed and developed an MITF dimerization-based AlphaScreen assay that sensitively and specifically mirrors the dimerization of MITF in vitro. Thus the goal of this study is to identify MITF inhibitors by high throughput screening (HTS) of large compound libraries. Compounds disrupting MITF dimerization suppress the DNA-interacting ability of MITF, provide a novel and effective approach for exploring MITF inhibitors with the potential to serve a role in melanoma treatment.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Center Affiliation: The Scripps Research Institute
Assay Provider: Thomas Kodadek, The Scripps Research Institute
Network: Scripps Molecular Screening Center
Grant Proposal Number: 1R01 CA178315-02
Grant Proposal PI: Thomas Kodadek, The Scripps Research Institute
External Assay ID: MITF_INH_Alpha_1536_1X%INH PRUNç

g. Reactivate the silenced genes in cancer cells

i. MBD2

Description

Of all the somatic genome changes that accumulate during the pathogenesis of human cancers, only changes in DNA methylation appear to occur consistently (virtually all cases), to arise early (first appearing in preneoplastic lesions), and to be potentially reversible (the DNA sequence remains intact) (1-4). One such change in DNA methylation, increased CpG dinucleotide methylation at CpG islands encompassing the transcriptional regulatory regions of many genes, leads to the transcriptional "silencing" of critical cancer genes (2, 5-6). CpG island hypermethylation has been reported to inhibit gene transcription by interfering with the binding and/or function of transcriptional trans-activators, or by recruiting 5-meCpG-binding domain (MBD) family proteins capable of mediating transcriptional repression (7). As many as 500 or more genes are epigenetically "silenced" in most human cancers. Two MBD family proteins have been implicated in the silencing of genes carrying abnormally hypermethylated CpG island sequences, MECP2 and MBD2. MBD2 binds 5-meCpG-DNA and is a component of a 1 MD transcription repression complex that also contains a chromatin remodeling complex subunits, histone-binding proteins, and a helicase/ATPase domain (8). Evidence suggests that MBD2-containing complexes are responsible for transcriptional repression accompanying somatic hypermethylation at pi-class glutathione S-transferase 1 (GSTP1), the most common genome change yet reported for prostate cancer, and a common alteration in breast and liver cancers (9-11). The goal of this project is the discovery and characterization of small molecule inhibitors of epigenetic gene silencing mediated by MBD2 and thus to identify compounds that will be able to reactivate the silenced genes in cancer cells, restoring gene function.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Bill Nelson
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R03 MH098712-01
Grant Proposal PI: Bill Nelson
External Assay ID: MBD2-CPGDNA_INH_TRFRET_1536_1X%INH PRUN

h. Colon Cancer

i. Phenotypic screens: 8 CoCa cell line screens at ChEMBL

2. Cell Mechanisms

a. Autophagy

i. Autophagy imaging assay

Description

Macroautophagy (referred to as autophagy herein) is a fundamental biological phenomenonthat significantly impacts energy metabolism and intracellular homeostasis. Morphologically, autophagy is defined by electron microscopy as a double membrane structure (autophagosome) that captures cytoplasm, subcellular organelles, or even intracellular pathogens, which eventually fuses with the lysosome, leading to the degradation of its content. Research in the last decade has defined a core machinery of autophagy, which involves two conjugation systems: the Atg5-Atg12-Atg16 and the Atg8-PE. Because of its rapid translocation from the cytosol at the initiation stage to the autophagosomal membranes and its continuous presence in the vacuole until degradation, Atg8, or a mammalian homolog, LC3B, has become favorable molecular marker for autophagy, particularly in mammalian cells, in which no other markers can be monitored in a real time fashion. Nonetheless, many fundamental issues are still unresolved regarding the molecular mechanisms of autophagy.
Studies in mammalian cells have now accumulated compelling evidence that autophagy is intimately involved in the pathogenesis in such prevailing diseases as cancer, neurodegenerative diseases and intracellular microbial infections. Modulation of autophagy has resulted in changes in disease course, which leads to the hope that this could open up new therapeutic venues. Unfortunately, there is a lack of good compounds or drugs that could be used clinically. Most of the agents that are known to modulate autophagy are indirectly affecting the autophagy machinery, or have non-specific targets. This issue is further compounded by our incomplete understanding of the molecular mechanisms of autophagy in different settings regarding its initiation and regulation, which renders the search for compounds against specific targets difficult.

We have developed a cell-based high-content screen that can be performed reproducibly in microtiter plate-based format with robotic liquid handling. The assay is a high-content imaging assay based on changes in the cellular localization of GFP-LC3B from a diffuse cytosolic to a membrane-associated, punctate pattern. All experiments were performed in collagen-coated 384 well microplates following the assay protocol below. Image acquisition and analysis were done on the ArrayScan VTi platform (ThermoFisher Cellomics).

Source: University of Pittsburgh Molecular Library Screening Center
Grant Number: R03 MH081275

ii. ATG4B

Description

Autophagy is an evolutionarily conserved process whereby cells catabolize damaged proteins and organelles for purposes of generating substrates for sustaining ATP production during times of nutrient deprivation. The autophagic process involves membrane vesicles engulfing cytosol and organelles, delivering their contents to lysosomes for digestion. The genes responsible for autophagy have been identified, largely through genetic analysis of yeast, Saccharomyces cerevisiae, and are conserved in mammals, plants, and essentially all eukaryotes. While autophagy is critical for cell survival in the context of nutrient deprivation, circumstances where autophagy and cell death occur in tandem have been identified, especially in tumor biology. For example, apoptosis-defective tumors are reliant on autophagy to survive metabolic stress. Also, autophagy inhibitors can be used to induce acute necrotic cell death, which may be facilitated by concurrent proteasome inhibition, enabling tumor eradication. In the adjuvant setting, and after elimination of a large proportion of the tumor by radiation and chemotherapy, the remaining cells can reside in a disrupted and stressed environment, susceptible to inhibition of the autophagy survival mechanism. Tumor cells in the process of metastasis can be similarly vulnerable.

Chemical modulators of autophagy are essentially non-existent, with the only available agent, 3-methyladenine, requiring millimolar concentrations to inhibit class III phosphatidyl inositol kinases (PI3Ks) involved in autophagy. A need exists for chemicals that target specific components of the autophagy machinery -- both for use as research tools for addressing questions about the role of autophagy in diseases such as cancer.

Autophagins are a class of cytosolic cysteine proteases required for autophagy. The human genome contains four independent genes encoding the Autophagins, including ATG4A, 4B, 4C, and 4D. These proteases cleave Gly120 site of LC3 (ATG8) to promote its conjugation with phosphatidylethanolamine (PE) by a ubiquitin-like system, which is required for autophagosome formation and that participate in targeting these vesicles to lysosomes for fusion and degradation of their contents. Autophagins also promote deconjugation of LC3-PE to liberate LC3 from membrane at or before the final stage of fusion between autophagosome and lysosomes, suggesting that deconjugation of LC3-PE is required for the fusion.

In this assay, a High Throughput Screening (HTS) assay based on fluorescence intensity is utilized to screen for compounds that inhibit Autophagin 1 (ATG4B). The HTS assay utilizes a cleavable form of Phospholipase A2 (PLA2), which is expressed as a fusion protein with the Autophagin substrate LC3/ATG8 appended to its N-terminus. The addition of sequences to the N-terminus of PLA2 inhibits the activity of this enzyme. Cleavage by proteases removing the N-terminal extension then restores enzyme activity, constituting the basis for a protease assay. Together, these efforts will result in validated chemical probes for studying the autophagy in a variety of biological settings.

Data Source: Sanford-Burnham Center for Chemical Genomics (SBCCG)
Source Affiliation: Sanford-Burnham Medical Research Institute (SBMRI, San Diego, CA)
Network: NIH Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Number: 1 R03 MH090871-01.
Assay Provider: Dr. John C. Reed, Sanford-Burnham Medical Research Institute, San Diego CA

b. Cytotoxicity

i. cell growth assays at ChEMBL

A number of cytotoxicity assays on different cell lines stored ibn ChEMBL repository.

c. Lipid transport

i. Scarb1

Description
The overall goal of this project is to identify inhibitors and use small molecules to analyze the mechanism of the HDL receptor called Scavenger Receptor, class B, type I (SR-BI). SR-BI was the first high density lipoprotein (HDL, "good" cholesterol) receptor identified. It controls the structure and composition of plasma HDL, and levels and fates of HDL cholesterol, including delivery to the liver and steroidogenic tissues. SR-BI binds HDL and functions i) as a cell surface transporter to move cholesterol or its esters into or out of cells

and ii) as a signaling receptor to control cell function. It also can interact with and/or transport a plethora of other ligands.

Developing tools to permit the analysis of SR-BI function and mechanism of action (see below), as well as the manipulation of SR-BI's activity in vitro and in vivo, will have significant impact on our understanding of diverse areas of physiology and pathophysiology of considerable medical importance. Indeed, recent disappointments in attempts to develop HDL-focused pharmaceutical agents with other molecular targets highlight the importance of developing a deeper and broader understanding of all aspects of HDL metabolism, including those mediated by SR-BI. Pharmacologic agents that modulate SR-BI activity have potential clinical applicability (inhibitors that block cellular uptake of pathogens (e.g., HCV) and activators that stimulate atheroprotective lipoprotein metabolism), as well as their use as probes to understand the molecular mechanisms underlying SR-BI's multiple activities. Given the central role of SR-BI in lipid transfer and metabolism, both activators and inhibitors of SR-BI function will be useful tools to further probe the mechanism of SR-BI.

Primary Collaborators:
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Miao Yu,MIT,miaoyu@mit.edu

d. Epigenetics

i. bromodomain AZ2B

Description

BAZ2B (bromodomain adjacent to zinc finger domain, 2B) belongs to a family of ubiquitously expressed bromodomain containing proteins, which biological function has not yet been elucidated. However, it is suggested that BAZ2B has a similar function as the Drosophila Acf1 protein which regulates nucleosome mobilization through the ATP-dependent chromatin remodelling factor ISWI, resulting in alteration in the translational position of the histone and hence transcriptional regulation. The interaction of BAZ2B with ISWI mediated by the BAZ1 motif has recently been described.

There is a need for chemical probes to allow the elucidation of the roles of this protein in health and disease and hence a quantitative high-throughput screen was developed. The protocol is based on the AlphaScreen (PerkinElmer) displacement assay developed by NCGC, but uses the peptide ligand Biot-H3K14Ac, a high affinity binding partner for BAZ2B, in an AlphaScreen format.

Although AlphaScreen has significant advantages with its utility in a variety of epigenetic target assays, the primary screening data provided in this deposition should be used with caution due to the prevalence of screening artifacts. The top compound concentration tested was 110uM in this assay, which increases number of actives and potential artifacts. An AlphaScreen counterscreen is highly recommended to be run against any putative actives to eliminate non-specific artifacts. Alternatively, one can use other AlphaScreen assays in PubChem to filter out promiscuous hits.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
Structural Genomics Consortium (SGC)

NIH Grant: 5U54 MH084681-02

ii. E92 histone-lys N-methyltransferase 2

Description

Methylated lysines, on the N-terminal tails of histone proteins serve as epigenetic markers to recruit factors that can then modify local chromatin structure to lead to functional consequences. G9a and other members of the SUV39 family of the SET domain-containing superfamily of histone lysine methyltransferases (HMT) have been identified to specifically methylate Lys9 of histone H3 (H3K9). G9a catalyzes the mono- and di-methylation of H3K9 in mammalian euchromatic regions, where the resulting H3K9me2 is indicative of transcriptional repression. Hence, G9a has been recognized as a potential drug target for several human diseases, including cancer. The inhibition of G9a will result in transcriptional activation and work synergistically with DNA methyltransferase and histone deacetylase inhibitors, to kill cancer cells.

This qHTS assay for identification of G9a inhibitors is a chemiluminescence based AlphaScreen (PerkinElmer). Methylation of biotinylated-histone peptide is measured through specific antibody-based detection, in conjunction with streptavidin-coated donor and anti-IgG antibody-coated acceptor beads. The method is particularly well suited for detection of inhibitors acting by the desired histone peptide competitive mechanism and is applicable to testing other HMTs.

Although AlphaScreen has significant advantages with its utility in a variety of epigenetic target assays, the primary screening data provided in this deposition should be used with caution due to the prevalence of screening artifacts. An AlphaScreen counterscreen is highly recommended to be run against any putative actives to eliminate non specific artifacts. Alternatively, one can use other AlphaScreen assays in PubChem to filter out promiscuous hits.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
Structural Genomics Consortium (SGC)
NIH Grant: 5U54 MH084681-02

iii. Jmjd2a

Description

JMJD2A is a jumonji-domain-containing lysine demethylase that uses Fe(II) together with 2-OG to effect oxidative demethylation of histones. Methylation is one of many post-transcriptional modification that affect epigenetic transcription. Hence, cross-talk between histonemarks and histone modifying enzymes exists, whereby enzymes that transfer/remove histone marks also contain reader domains. JmjD2A has been shown to specifically bind to trimethyl H3K4 and H4K20 marks It is proposed that the functions of JmjD2A binding to the histones, is to maintain marks after they are installed and to spread the modifications by positioning the enzyme for methylation of adjacent regions, establishing a self-propagating cycle for rapid methylation over a large area of chromatin. Due to the combinatorial nature of the histone code and the cross-talk between regulatory proteins, the value of a probe directed against a given target can be enhanced by possessing knowledge about the probes' interactions with additional epigenetic proteins. Thus, small molecule targeting of the JMJD2A-tudor domain interaction with its target methylhistone mark is expected to enable sophisticated experiments probing the fine regulatory pathways leading to selective demethylation of a given methyllysine locus based on the methylation

state of adjacent histone marks. A quantitative high throughput AlphaScreen has been developed to identify small molecule inhibitors based on a protocol published by NCGC and SGC.

Although AlphaScreen has significant advantages with its utility in a variety of epigenetic target assays, the primary screening data provided in this deposition should be used with caution due to the prevalence of screening artifacts. The top compound concentration tested was high (>100uM) in this assay, which increases number of actives and potential artifacts. An AlphaScreen counterscreen is highly recommended to be run against any putative actives to eliminate non-specific artifacts. Alternatively, one can use other AlphaScreen assays in PubChem to filter out promiscuous hits.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
Structural Genomics Consortium (SGC)
NIH Grant: 5U54 MH084681-02

3. Immune

a. Fibrosis

i. relaxin PMs

Description

The relaxin hormone is involved in the variety of biological functions in normal tissues and diseases. The role of relaxin is well-established in female reproduction and parturition, mammary gland and endometrial development, maintenance of myometrial quiescence during pregnancy. Relaxin signaling through its G protein-coupled receptor (GPCR) RXFP1 results in ECM remodeling through regulation of collagen deposition, cell invasiveness, proliferation and overall tissue homeostasis. Significantly, the therapeutic effects of relaxin in the treatment of renal, cardiac, skin, lung fibrosis, inflammation, and wound healing in animal models are well-established. Recombinant human relaxin (rhRlx) is currently being tested in clinical trials as a protective agent in congestive heart failure, in treating severe preeclampsia, and as an anti-fibrotic agent in various organs. It was demonstrated that the increased relaxin/RXFP1 signaling is associated with more aggressive forms of prostate, breast, endometrial and other cancers.

Upon relaxin binding RXFP1 activation leads to the activation of adenylate cyclase (AC) via G?s. cAMP will activate PKA, which phosphorylates many signaling proteins. Thus, detection of cAMP increase is an easy and reliable indication of relaxin receptor activation. To screen for antagonists of the relaxin receptor, a HEK293T cell line stably transfected with RXFP1 was used. RXFP1 activation by relaxin in presence of various concentrations of tested compounds was assayed by changes in cAMP levels as detected with a time-resolved fluorescence energy transfer (TR-FRET) cAMP detection kit.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production Centers Network [MLPCN]
MLPCN Grant: R03 MH085705-01A1
Assay Submitter (PI): Alexander Agoulnik

NCGC Assay Overview:

ii. relaxin agonists

Description

The relaxin hormone is involved in the variety of biological functions in normal tissues and diseases. The role of relaxin is well-established in female reproduction and parturition, mammary gland and endometrial development, maintenance of myometrial quiescence during pregnancy. Relaxin signaling through its G protein-coupled receptor (GPCR) RXFP1 results in ECM remodeling through regulation of collagen deposition, cell invasiveness, proliferation and overall tissue homeostasis. Significantly, the therapeutic effects of relaxin in the treatment of renal, cardiac, skin, lung fibrosis, inflammation, and wound healing in animal models are well-established. Recombinant human relaxin (rhRlx) is currently being tested in clinical trials as a protective agent in congestive heart failure, in treating severe preeclampsia, and as an anti-fibrotic agent in systemic sclerosis.

Upon relaxin binding RXFP1 activation leads to the activation of adenylate cyclase (AC) via Gs. cAMP will activate PKA, which phosphorylates many signaling proteins. Thus, detection of cAMP increase is an easy and reliable indication of relaxin receptor activation. To screen for agonists of the relaxin receptor, a HEK293T cell line stably transfected with RXFP1 was used. RXFP1 activation was assayed by changes in cAMP levels as detected with a time-resolved fluorescence energy transfer (TR-FRET) cAMP detection kit.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production Centers Network [MLPCN]
MLPCN Grant: R03 MH085705-01A1
Assay Submitter (PI): Alexander Agoulnik
NCGC Assay Overview:

iii. NCF1

Description

Oxidative stress (the excess production of cellular oxidizing substances) is a central component in many diseases. Reactive oxygen species (ROS) produce oxidative stress that plays a central role in inflammation in general, and in the tissue damage and abnormal cell growth and fibrosis associated with many diseases. ROS-associated diseases often represent chronic conditions, and are frequently associated with tissue damage, fibrosis, and in some cases probable genetic damage. Numerous examples of ROS-associated diseases have been identified, and include diseases and pathologies of the cardiovascular system, nervous system, endocrine system, respiratory system, excretory system, and others. Nox enzymes provide most of the ROS in these conditions. Noxs, particularly Nox2 and Nox1, confer the major source of oxidative stress in these diseases, and inappropriate activation of these enzymes causes inflammation and tissue damage in these conditions. Recent studies using transgenic and knockout mouse models validate these enzymes as new targets for the development of CVD drugs. Such inhibitors will comprise a new class of drugs useful in prevention and treatment of cardiovascular and other disease.

To discover novel small molecule inhibitors of Noxs, a highly sensitive fluorescence polarization (FP) based assay was designed and optimized in 1536-well format. In this assay, the interaction of p47phox protein with a fluorescently labeled peptide derived from p22phox was used as a model system. p47phox is an essential regulatory subunit for activity of Nox2. The homologous regulatory subunit for Nox1 is NOXO1. Both enzymes are membrane-associated and bind in the membrane to a second subunit, p22phox. p22phox

has a C-terminal region containing a proline-rich domain (PRD) that provides a key binding site for either NOXO1 or p47phox. A simple 1-step mix-and-measure method was achieved for analyzing p47phox protein and p22phox interaction. The p47phox/p22phox FP assay is highly stable and has achieved a robust performance in a 1536-well uHTS format with a demonstrated signal-to-noise ratio of above 10 and a Z## factor of above 0.6. Because of its simplicity and high sensitivity, this assay is used for ultra high-throughput screening in 1536 well format of small molecules inhibitors of NADPH oxidases (Noxs).

NIH Molecular Libraries Screening Centers Network [MLSCN]
Emory Chemical Biology Discovery Center in MLSCN
Assay provider: Susan Smith, Emory University
MLSCN Grant: MH083234-01

iv. MSRA

Description

Oxidative damage, resulting from the production of reactive oxygen species (ROS) within cells, is believed to be a major factor in age-related diseases and the aging process. One of the mechanisms by which this damage occurs is via oxidation of methionine residues to methionine sulfoxide (Met(O)) derivatives in cellular proteins, which can lead to protein inactivation. These Met(O) species can be repaired/reduced by the thioredoxin (Trx)-dependent action of Methionine sulfoxide reductase A (MsrA). MsrA can reduce both free and protein-bound Met(O), and is highly expressed in oxidant-sensitive tissues such as kidney, neurons, liver, retinal epithelial cells, and macrophages. Each round of methionine oxidation and reduction by the MsrA system destroys one equivalent of ROS. Importantly, the action of MsrA has been shown to prevent irreversible oxidative protein damage and extend life span of both flies and yeast. As a result, the identification of compounds that modulate MsrA activity could have therapeutic value for cardiovascular, neurodegenerative, lung, and eye diseases involving oxidative damage. Similarly, because MsrA is found in virtually all species, and the catalytic mechanism has been elucidate , the identification of chemical tools that modulate MsrA would help elucidate its function and activation in cells, and may lead to useful tools to extend lifespan and reduce aging-related diseases.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Herbert Weissbach, Florida Atlantic University
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1R03DA032473-01
Grant Proposal PI: Herbert Weissbach, Florida Atlantic University
External Assay ID: MSRA_ACT_ABS_1536_1X%ACT

v. Lung Fibroblast Proliferation

Description

This assay was designed to interrogate the MLSMR for compounds that inhibit fibroblast proliferation. Compounds that are inactive in this assay, but active in the HUVEC assay will be evaluated further as potential probes for angiogenesis research. Conversely, compounds that selectively inhibit the proliferation of fibroblasts may have utility for other areas of research such as the study of mechanisms involved in tumor desmoplasia or diseases involving fibrosis.

Southern Research Molecular Libraries Screening Center (SRMLSC)

Southern Research Institute (Birmingham, Alabama)
NIH Molecular Libraries Screening Centers Network (MLSCN)
Assay Provider: Dr. Zhican Qu, Southern Research Institute
Award: X01-MH079851-01

vi. SMAD3

Description

Transforming growth factor beta (TGF-Beta) regulates a variety of processes in mammalian cells, including proliferation, apoptosis, cell migration and extracellular matrix production. Aberrant increases in TGF-Beta signaling have been implicated in several pathological conditions including cancer and fibrosis. Inhibition of TGF-Beta signaling is an important tool in elucidating the multiple biological functions of TGF-Beta and is of significant interest as a potential therapeutic strategy in fibrotic diseases and several advanced cancers.
Smad proteins mediate cellular responses to TGF-Beta. TGF-Beta alters cellular gene expression and cell behavior by binding and activating the Type II and Type I serine kinase receptors on the cell membrane. Activated Type I receptor phosphorylates Smad2 and Smad3, which form heterodimeric or heterotrimeric complexes with Smad4 that accumulate in the cell nucleus. The Smad2-Smad4 and Smad3-Smad4 protein complexes bind over 20 different nuclear proteins including DNA-binding proteins, transcription activators and transcription repressors. The development of therapeutic strategies that target TGF- Beta signaling depends on selectively affecting the pathological actions of TGF-Beta with minimal effects on its normal functions. Disruption of only one binding site on Smad should block binding of some but not all Smad binding partners, thereby potentially interfering with only a subset of the gene expression responses mediated by Smad complexes. The identification of peptide aptamers with selective effects on TGF- Beta gene expression suggests that selective disruption of Smad transcriptional complexes might provide selective inhibition of some but not all TGF-Beta responses (Cui, Q., et al, 2005; Oncogene, 24: 3864-74). Inhibition of Smad function will be an important research tool in elucidating the multiple biological functions of TGF-Beta.
To screen for small molecular inhibitors that disrupt Smad protein-protein interactions, a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) assay was developed to measure the binding between Smad3 protein and the twenty-four amino acid FoxH1 Smad interaction motif (SIM) in a 384-well format. Smad3 with a 6xHis tag was indirectly labeled with a europium chelate through a europium conjugated anti-6XHis antibody. Peptide (FoxH1 SIM) was synthesized with an N-terminal Dy647 (MoBiTec) tag. Europium and Dy647 comprise a fluorescence energy transfer pair. Interaction of Smad3 protein with hFoxH1 peptide brings two conjugated fluorophores into proximity, leading to an energy transfer from europium to Dy647 and the generation of FRET signal. FRET signal is detected in an Analyst HT plate reader (Ex 340 nM, Em615 nM and Em665 nM) and expressed as FRET signal ratio (F665nm / F620nm * 104). The assay is robust with a consistent Z' factor of 0.6-0.8 in a 384-well plate format and is used for the screening of the NIH/DPI library of 86,106 compounds for Smad3/hFoxH1 inhibitors.

NIH Molecular Libraries Screening Centers Network [MLSCN]
Emory Chemical Biology Discovery Center in MLSCN
Assay provider: F.M. Hoffmann, University of Wisconsin-Madison
MLSCN Grant: 1R21NS057002-01

vii. SMAD3 (TFG-beta)

Description

TGF-b is a main component in the TGF-b signaling pathway which plays diverse roles in cellular and development pathways. TGF-b is the mediated by the transcription factors Smads. Although the Smad-dependent pathway is the primary canonical TGF-b signaling node, TGF-b1 can also activate alternative signaling pathways, including those involving MAPK (ERK, JNK and p38). This interaction may mediate or enhance Smad-dependent responses, or can exert Smad-independent effects. The complexity of this signaling cascade allows the TGF-b superfamily to perform unique, overlapping or redundant functions. We believe that targeting the TGF-beta pathway at the Smad-transcription factor level may eliminate the consequences of disrupting the entire pathway and offer specificity without affecting other signaling pathways. Smad3 is the primary transducer of TGF-b's signals and Smad3 regulates many functions attributed to TGF-b signaling. We hypothesize that Smad3 inhibitors will selectively eliminate Smad3-specific TGF-beta signals without undesired off-target effects. We aim to identify Smad3-small molecule antagonists using a quantitative high throughput screening (qHTS) approach.

A high-throughput assay was developed to screen the NIH Molecular Libraries Small Molecule Repository (MLSMR). This is a cell based assay, where TGF-b is tagged with GFP. A counterscreen is run that looks at cytotoxicity (please see summary AID).

MH087449NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH087449
Assay Submitter (PI): Sushil Rane, National Institute of Diabetes and Kidney Diseases

viii. ALR inhibitors

Description

The goal of this project is to develop small molecule modulators that affect the mitochondrial sulfhydral oxidase pathways by using purified components, specifically ALR, in an in vitro assay.

Defects in mitochondrial assembly impact a wide range of diseases from degenerative muscle and neural diseases to cancer. The mitochondrion is not only important for the production of energy but plays an important role in other aspects such as intermediary metabolism and signaling. The mitochondrion contains an inner membrane and outer membrane that separate the matrix from the intermembrane space. The intermembrane space is an important compartment because it houses molecules such as cytochrome c and apoptosis inducing factor (AIF) that are released during apoptosis. Recently, the intermembrane space has been implicated in diseases such as Parkinson's disease by the presence of the kinase PINK1. In addition, we continue to make new discoveries about the function of this compartment. Specifically, we have identified a new import pathway in the intermembrane space that is redox regulated, indicating that oxidation-reduction reactions are unexpectedly housed in this compartment. Key components include the sulfhydryl oxidase Erv1 and cytochrome c, which form a complex in the intermembrane space. A recent study has demonstrated a role of ALR, the human Erv1 homolog, in Autosomal-Recessive Myopathy. Both Erv1 and ALR belong to a class of FAD dependent sulfhydryl oxidases that have essential roles in the mitochondrion.

Screen for inhibitors of purified, recombinant ALR to oxidize the generic substrate DTT. Oxidation of DTT by ALR produces hydrogen peroxide, which was detected using horseradish peroxidase (HRP) and luminol. A solution of ALR and HRP was added to plates containing compounds, followed by the addition of DTT. The plates were incubated for 2 hours prior to the addition of the luminol solution. After a minute incubation, plates were read to detect the flash luminescence signal.

Primary Collaborators: Carla Koehler,UCLA,Los Angeles,CA,koehlerc@chem.ucla.edu,310-794-4834. Deepa Dabir,UCLA,Los Angeles,CA,deepad@chem.ucla.edu
Samuel Hasson,UCLA,Los Angeles,CA,hassons@gmail.com

b. COPD

i. Nfr2 activators

Description

Many diseases have some form of oxidative stress injury and ties to inflammation, causing a host of problems for the patient. The antioxidant response element (ARE) plays an important role in alleviating the harmful effects of oxidative stress. The antioxidant response element (ARE) is a transcriptional regulatory element involved in the activation of genes coding for a number of antioxidant proteins and detoxifying enzymes. These enzymes work in concert to protect tissues from oxidative insults and chemical toxicities in human hepatocytes and immune cells. The protective effects of ARE activation are primarily triggered through Nrf2 (NF-E2-related factor) binding to and activating AREs. It is known that Nrf2 controls the production of over 250 antioxidant and detoxification proteins (Hu et al., 2006), thereby protecting tissues by increasing cellular antioxidant content and suppressing inflammatory signaling pathways. The transcription factor Nrf2 is a central link between oxidative chemicals, such as phenolic antioxidants and electrophilic compounds, and the activation of ARE. Nrf2 levels are constitutively low as a consequence of its interaction with Keap1, which targets its degradation. Electrophiles react with key cysteine residues in Keap1, releasing Nrf2 and allowing its translocation to the nucleus. Once within the nucleus, Nrf2 complexes with coactivators such as p300, and binds to the AREs to induce gene transcription of cytoprotective enzymes, resulting in the prevention of toxicity. Activation of Nrf2 protects tissues by increasing cellular antioxidant content and suppressing inflammatory signaling pathways. Identifying novel and potent Nrf2 activators by using the AREc32 cell line to detect ARE activation by small molecules will lead to the identification and development of probes to study protective pathways in multiple tissues. These specific probes are essential to study the ARE pathway, and eventually to determine whether this pathway does activate genes that could protect against a host of diseases, including cardiovascular diseases, obesity, diabetes, Alzheimer's, and Parkinson's disease.

This project's aim is to identify novel and potent Nrf2 activators by using the AREc32 cell line to detect ARE activation by small molecules, which will lead to the identification and development of probes to study the ARE pathway.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: DK081461
Assay Submitter (PI): Curtis Klaassen, University of Kansas Medical Center

ii. Antioxidant Response Element

Description

Oxidative stress has been implicated in the pathogenesis of a variety of diseases ranging from cancer to neurodegeneration. The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress. The CellSensor ARE-bla HepG2 cell line (Invitrogen) can be used for analyzing the Nrf2/antioxidant response signaling pathway. Nrf2 (NF-E2-related factor 2) and Nrf1 are transcription factors that bind

to AREs and activate these genes. The CellSensor ARE-bla Hep G2 cell line contains a beta-lactamase reporter gene under control of the Antioxidant Response Element (ARE) stably integrated into HepG2 cells. The activation of the report gene under culture conditions can be detected by fluorescence intensity. This cell line has been used to screen the Tox21 10K compound library to identify agonists that induce oxidative stress. The cytotoxicity of the Tox21 compound library against the ARE-bla cell line was tested in parallel by measuring the cell viability using CellTiter-Glo assay (Promega, Madison, WI) in the same wells. The compounds were also tested for auto fluorescence that may interfere with the biological target readout resulting in potential false positives and/or negatives.

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

iii.

c. Autoimmune

i. Rorc transcriptional activity

Description

The retinoic acid-related orphan receptor (ROR) gamma is a transcription factor that has a central role in the differentiation of Th17 cells, a subset of T helper cells that secrete the inflammatory cytokines IL-17, IL-17F, and IL-22. Th17 cells have been implicated in graft versus host disease, autoimmune disease and asthma. ROR gamma is induced in naive T helper cells in the presence of TGF-beta combined with IL-6, IL-21, or IL-23, and thereafter directs the expression of the Th17 lineage cytokines. To study the mechanism of action of ROR gamma, an insect cell-based assay was screened. This Drosophila Schneider cell line was stably transfected with two vectors: a gene expressing a fusion of the Gal4 DNA binding domain and ROR gamma transactivation domain under the control of the metallothionine promoter and a Photinus luciferase reporter regulated by the Gal4 binding site enhancer, UAS. The addition of copper to the medium induced expression of the Gal4-ROR gamma fusion that subsequently induced the UAS-luciferase reporter. Small molecule inhibitors of ROR gamma activity are detected by a decrease in luciferase reporter activity.

NIH Molecular Libraries Probe Production Network [MLPCN]
NIH Chemical Genomics Center [NCGC]
MLPCN Grant: R03 DA026211-01
Assay Provider: Dan Littman, New York University

ii. Rorc signalling pathway

Description

Nuclear steroid hormone receptors are involved in many biological processes including inflammatory responses, cell proliferation, and apoptosis. Environmental factors or compounds disrupting these nuclear receptor pathways and gene expression may promote susceptibility to immunotoxicants and autoimmune diseases. One important group of these expression factors are the retinoid-related orphan receptors. To assess compounds that may

interfere with the retinoid-related orphan receptor gamma (ROR-gamma) transcription factor, a cell-based luciferase assay was used.

The CHO cell-based assay utilizes a TET-inducible ROR expression factor and ROR response element-LUC reporter gene. Reporter gene activity was induced using Doxycycline Hyclate and measured after 16 hr incubation. Tet-on ROR gamma CHO cells were used to screen against the Tox21 10K compound library for ROR gamma antagonists by quantification of decreased reporter gene activity in terms of decreased luminescence.

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

d. Immunomodulation

i. CTL granule exocytosis

Description

Target cell killing by cytotoxic T lymphocytes (CTLs), critical for the immune response to viruses and tumors, is involved in transplant rejection and contributes to autoimmune disease pathogenesis. A key mechanism CTLs use to kill target cells is secretion of cell-killing agents from specialized lysosomal lytic granules. Screening a small molecule library for compounds that inhibit lytic granule exocytosis would 1) serve as an assay for blockers of the known signals that control the process; 2) reveal the presence of and define chemical probes for as-yet-unknown signals and 3) lead to novel immunomodulatory drugs that could enhance our ability to transplant organs and stem cells and to treat autoimmune disorders.

CTL granule exocytosis can be monitored using fluorescently-labeled antibodies to detect the externalization of lysosome-associated membrane protein 1 (LAMP-1) from lytic granules to the plasma membrane in a simple no-wash assay protocol suitable for flow cytometry. We plan to capitalize on this simple assay together with the unique HTS flow cytometry capabilities at the University of New Mexico Center for Molecular Discovery (UNMCMD) to establish a phenotypic screen and to discover chemical probes that inhibit granulocyte exocytosis.

Assay Support: NIH R21 NS066462
A high-throughput screen of lytic granule exocytosis
PI: Adam Zweifach, Ph.D.
PI Affiliation: Department of Molecular and Cell Biology, University of Connecticut
Screening Center PI: Larry Sklar, Ph.D. / UNMCMD
Screening Lead: Mark Haynes, Ph.D.
Assay Implementation: Bruce Edwards, Ph.D., Matthew Garcia, Mark Carter, M.S.
UNM Cheminformatics: Oleg Ursu, Ph.D.
Chemistry: Vanderbilt Specialized Chemistry Center for Accelerated Probe Development
Vanderbilt Specialized Chemistry Center PI: Craig Lindsley, Ph.D.
Vanderbilt Chemistry Lead: Michael Wood, Ph.D.

ii. STAT1 activation

Description

The signal transducer and activator of transcription (STAT) family of transcription factors transduce signals from a variety of extracellular stimuli and are important mediators of inflammation, cell survival, differentiation, and proliferation. STATs are activated in response to growth factors, cytokines, and G-CSF binding to cell surface receptor tyrosine kinases. In resting cells STATs are inactive in the cytoplasm. In response to stimuli, STATs are phosphorylated by the Janus-activated kinases (Jaks), which induces STAT dimerization and nuclear translocation, where STATs bind to specific enhancer elements in target genes. Although structurally similar, members of the STAT family (STATs 1, 2, 3, 4, 5a, 5b, and 6) possess diverse biological roles. For example, STAT1 activation is pro-inflammatory and anti-proliferative, while STAT3 activation is anti-inflammatory and pro-apoptotic. STAT1 is largely responsible for mediating the effects of IFN-gamma, while STAT3 is predominantly involved in IL-6 signaling. STAT1 induces expression of genes that inhibit the cell cycle, and thus STAT1 is considered to have tumor suppressor properties. Currently available STAT1 modulators mediate modest effects on STAT-induced transcription, act indirectly by targeting JAK or other kinases activities, or are associated with adverse hematologic or gastrointestinal side effects. The liabilities of the current state of the art for STAT1 modulators necessitate the discovery of higher affinity probes. Due to the diverse roles and potent phenotypes associated with STAT signaling, the identification of selective potentiators of STAT1 activity may lead to pharmacological tools for cancer, wound healing, and inflammatory diseases.

Source (MLSCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: David Frank
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number: 1 X01 MH079826-01
Grant Proposal PI: David Frank
External Assay ID: STAT1_POT_LUMI_1536_%ACT

iii. STAT3 activation

Description

The signal transducer and activator of transcription (STAT) family of transcription factors transduce signals from a variety of extracellular stimuli and are important mediators of inflammation, cell survival, differentiation, and proliferation. STATs are activated in response to growth factors, cytokines, and G-CSF binding to cell surface receptor tyrosine kinases. In resting cells STATs are inactive in the cytoplasm. In response to stimuli, STATs are phosphorylated by the Janus-activated kinases (Jaks), which induces STAT dimerization and nuclear translocation, where STATs bind to specific enhancer elements in target genes (2). Although structurally similar, the seven STAT family member (STATs 1, 2, 3, 4, 5a, 5b, and 6) possess diverse biological roles. For example, STAT1 activation is pro-inflammatory and anti-proliferative, while STAT3 activation is anti-inflammatory and pro-apoptotic. STAT1 is largely responsible for mediating the effects of IFN-gamma, while STAT3 is predominantly involved in IL-6 signaling. STAT1 induces expression of genes that inhibit the cell cycle, and thus STAT1 is considered to have tumor suppressor properties. Studies show that STAT3 is activated in a majority of breast and prostate cancers, and that STAT3 inhibition using RNA interference or a dominant negative leads to reduced cell proliferation, survival, and wound healing. Blocking STAT3 interaction with the epidermal growth factor receptor (EGFR) using peptide aptamers has been shown to reduce tumor growth. Due to the diverse roles and potent phenotypes associated with STAT signaling, the identification of selective

potentiators of STAT3 activity may lead to pharmacological tools for cancer, wound healing, and inflammatory diseases.

Source (MLSCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: David Frank, Dana Farber Cancer Institute
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number: 1 X01 MH079826-01
Grant Proposal PI: David Frank
External Assay ID: STAT3_POT_LUC_1536_%ACT

iv. STAT3 inhibition

Description

The signal transducer and activator of transcription (STAT) family of transcription factors transduce signals from a variety of extracellular stimuli and are important mediators of inflammation, cell survival, differentiation, and proliferation. STATs are activated in response to growth factors, cytokines, and G-CSF binding to cell surface receptor tyrosine kinases. In resting cells STATs are inactive in the cytoplasm. In response to stimuli, STATs are phosphorylated by the Janus-activated kinases (Jaks), which induces STAT dimerization and nuclear translocation, where STATs bind to specific enhancer elements in target genes. Although structurally similar, the seven STAT family member (STATs 1, 2, 3, 4, 5a, 5b, and 6) possess diverse biological roles. For example, STAT1 activation is pro-inflammatory and anti-proliferative, while STAT3 activation is anti-inflammatory and pro-apoptotic. STAT1 is largely responsible for mediating the effects of IFN-gamma, while STAT3 is predominantly involved in IL-6 signaling. STAT1 induces expression of genes that inhibit the cell cycle, and thus STAT1 is considered to have tumor suppressor properties. Studies show that STAT3 is activated in a majority of breast and prostate cancers, and that STAT3 inhibition using RNA interference or a dominant negative leads to reduced cell proliferation, survival, and wound healing. Blocking STAT3 interaction with the epidermal growth factor receptor (EGFR) using peptide aptamers has been shown to reduce tumor growth. Due to the diverse roles and potent phenotypes associated with STAT signaling, the identification of selective modulators of STAT1 and STAT3 activity may lead to pharmacological tools for cancer, wound healing, and inflammatory diseases.

Source (MLSCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: David Frank, Dana-Farber Cancer Institute
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number: 1 X01 MH079826-01
Grant Proposal PI: David Frank
External Assay ID: STAT3_INH_LUMI_1536_%INH

e. Inflammation CASP COPD

i. IL-1b Inflammasome

Description

Inflammasomes are a set of intracellular multi-protein complexes that enable autocatalytic activation of inflammatory caspases and drive the innate immune response to counter harmful agents. Inflammasomes have been shown to contribute to the pathology of multiple autoinflammatory and autoimmune diseases, such as the Cryopryrin-Associated

Periodic Syndrome (CAPS) and Chronic Obstructive Pulmonary Disease (COPD). Patients with CAPS exhibit excessive production of IL-1B and IL-18 due to a gain-of-function mutation in NLRP3, while COPD patients have elevated levels of IL-1B as a downstream consequence of inflammasome activation. Anakinra has become the standard therapy for treating CAPS, but this drug has been shown to be inefficient because it relies on the stoichiometric scavenging of IL-1B rather than blocking inflammasome activation. Hence, the development of novel anti-inflammatory therapies for these chronic inflammatory diseases are of critical need.

A qHTS assay, based on AlphaLISA technology, was developed to screen the Molecular Libraries Small Molecule Repository (MLSMR) to look for novel inflammasome inhibitors. The assay detects IL-1B using AlphaLISA anti-IL-1B acceptor and donor beads. A decreased emission signal, indicating reduced IL-1B secretion, is the desired outcome for this assay.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH095513
Assay Provider: Shyam Biswal Ph.D., Johns Hopkins University

4. Infectious

a. Antiviral

i. SARS CoV

Machine Learning effort that uses different public experimental repositories to conduct a virtual screening on 1.8M ChEMBL molecules that identifies 64k predicted active compounds against SARS coronaviruses. All molecular interactions of these compounds with proteins with experimental records in ChEMBL are then explored to discover potential mechanisms of action. The site contains links leading to detailed analysis of more specific network graphs, compound structures and literature references supporting most relevant findings.
Click on specific tabs to review SARS-CoV biology, data sources, methodology, Evaluation of Machine Learning models and final predictive outcome.

ChEMBL and PUBCHEM DBs store a good amount of records of molecules interacting with SARS proteases and inhibiting viral proliferation in diverse phenotypic assays carried out with different coronavirus species.

PubChem stores data from 2 different HTS efforts on more than 300k molecules:

Summary of probe development efforts to identify inhibitors of the SARS coronavirus 3C-like Protease (3CLPro) A direct measurement of viral protease activity.

qHTS of Yeast-based Assay for SARS-CoV PLP. A yeast reporter assay of protease activity.

ChEMBL, at this moment contains >7k data points from >200 different assays in 12 coronavirus species. Most of these datasets come from viral proliferation or cell death

assays, although remains a small proportion of targeted models on proteases and polymerases.
ChEMBL SARS assays

And finally, a number of compounds reported to be active on coronavirus replication have been finally added to the model.

molName	publication
ASC-09	Coronavirus puts drug repurposing on the fast track – Nature
ritonavir	Coronavirus puts drug repurposing on the fast track – Nature
lopinavir	Coronavirus puts drug repurposing on the fast track – Nature
Darunavir	Coronavirus puts drug repurposing on the fast track – Nature
nelfinavir	HIV protease inhibitor nelfinavir inhibits replication of SARS …
K11777	Protease inhibitors targeting coronavirus and filovirus entry.
remdesivir	How to Conquer Coronavirus: Top 35 Treatments in …
Chloroquine	How to Conquer Coronavirus: Top 35 Treatments in …
danoprevir	How to Conquer Coronavirus: Top 35 Treatments in …
Favipiravir	How to Conquer Coronavirus: Top 35 Treatments in …
Brilacidin	How to Conquer Coronavirus: Top 35 Treatments in …
umifenovir	How to Conquer Coronavirus: Top 35 Treatments in …
chloroquine pp	How to Conquer Coronavirus: Top 35 Treatments in …
chloroquine HCl	How to Conquer Coronavirus: Top 35 Treatments in …
chloroquine p	How to Conquer Coronavirus: Top 35 Treatments in …
danoprevir Na	How to Conquer Coronavirus: Top 35 Treatments in …
(r)-chloroquine	How to Conquer Coronavirus: Top 35 Treatments in …

ivermectin	The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro.
aplidin	Antiviral activity of animal venom peptides and related compounds.

ii. HIV entry: Env-mediated Cell Fusion. Phenotypic.

Description

Although some inhibitors of HIV-1 entry exist, these are not suitable for use in a prophylactic setting because of limitations in potency, breadth and/or adaptability to a microbicide formulation. HIV-1 Envs expressed on the surface of cells can mediate the fusion of cells with target cells expressing the CD4 and CCR5/CXCR4 receptors, such as CEM lymphoblasts. We have established an assay that monitors the cell-cell fusion activity of HIV-1 Envs with a firefly luciferase readout. In our primary high-throughput screen (HTS), a modified HeLa cell line that inducibly expresses the Envs of a primary HIV-1 strain (HIV-1JRFL) will be co-cultivated with receptor-bearing target cells (CEM21) in the presence of the compounds to be screened. CEM21 expresses a Tet-activator responsive Luciferase reporter. This is a Tetracycline regulated system and when the Tet-activator from the Env-expressing cell line is transferred into the target cell upon successful fusion, the Luciferase reporter will be activated. Detection of Luciferase will occur using the SteadyGlo reagent (Promega, Madison, WI). Maraviroc, a known CCR5 antagonist, that blocks Env-mediated cell fusion will be used as a positive control.

Expected Outcome: Compounds that inhibit cell fusion will cause a decrease in Luciferase signal.

Source: Broad Institute
Grant Number: 1 R03 DA034601-01
Primary Collaborators: Alon Hirschhorn,Dana-Farber Cancer Institute,herschhorn@gmail.com; Joe Sodroski,Dana-Farber Cancer Institute,joseph_sodroski@dfci.harvard.edu

iii. Inhibitors of Hepatitis C Virus (HCV) . Phenotypic.

Description

Hepatitis C virus (HCV) infects about 200 million people in the world. Many infected people progress to chronic liver disease including cirrhosis with a risk of developing liver cancer. To date, there is no effective vaccine for hepatitis C. Current therapy based on interferon is only effective in about half of the patients and is associated with significant adverse effects. The fraction of people with HCV who can complete a successful treatment is estimated to be no more than 10 percent. Recent development of direct-acting antivirals against HCV, such as protease and polymerase inhibitors, is promising but still requires combination with peginterferon and ribavirin for maximal efficacy. In addition, these agents are associated with high rate of resistance and many have significant side effects.

Due to the lack of a culture system for infectious HCV, the search for new HCV drugs has been greatly hampered. Cell-based screen for HCV inhibitors in use today is based on the HCV replicon system, which only targets the RNA replication step of the viral lifecycle and does not encompass viral entry, processing, assembly and secretion. High-throughput screening (HTS) with an infectious HCV system would cover the complete spectrum of potentially druggable targets in all stages of HCV lifecycle, and would have more biological

relevance than other cell-based assays. Moreover, targeting several key processes in the viral life cycle may not only increase antiviral efficacy; more importantly, it may also reduce the capacity of the virus to develop resistance to the compound.

The goal of this project is to identify novel HCV inhibitors as new therapies for hepatitis C, using a highly sensitive and specific assay platform which is based on a HCV infectious cell culture system established in the laboratory and adapted for high-throughput HCV drug screen.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH095511
Assay Submitter (PI): Jake Liang, NIDDK

iv. A Cell Based HTS Approach for the Discovery of New Inhibitors of Respiratory syncytial virus (RSV) . Phenotypic.

Description

Assay Rationale and Summary: Currently, there are no commercially available vaccines to protect humans against Respiratory syncytial virus (RSV). RSV is associated with substantial morbidity and mortality and is the most common cause of bronchiolitis and pneumonia among infants and children under one year of age. Nevertheless, severe lower respiratory tract disease may occur at any age, especially among the elderly or among those with compromised cardiac, pulmonary, or immune systems. The existing therapies for the acute infection are ribavirin and the prophylactic humanized monoclonal antibody (Synagis from MedImmune) that is limited to use in high risk pediatric patients. The economic impact of RSV infections due to hospitalizations and indirect medical costs is greater than $ 650 million annually. The assay provider has developed and validated a 384-well cell-based assay that measures CPE induced in HEp-2 cells by RSV infection, using a luminescent-based detection system for signal endpoint. We anticipate that the proposed studies utilizing the Molecular Libraries Probes Production Network (MLPCN) HTS resources will generate multiple scaffolds targeting various junctures in the RSV viral lifecycle. These may be furthered developed into probes to construct novel single or combination therapeutics.

Southern Research's Specialized Biocontainment Screening Center (SRSBSC)
Southern Research Institute (Birmingham, Alabama)
NIH Molecular Libraries Probe Centers Network (MLPCN)
Assay Provider: Dr. William Severson, Southern Research Institute
Grant number: 1 R03 MH082403-01A1

v. hCMV. Phenotypic.

Description

NCGC Assay Overview: Infection with human cytomegalovirus (HCMV) continues to pose significant threats for pregnant women and immunocompromised hosts. HCMV causes serious morbidity and mortality in transplant recipients, and congenital HCMV is the leading infectious cause of mental retardation and deafness in children. In the 1990s, the annual costs associated with the disease burden of congenital CMV were estimated at around $2 billion. A HCMV vaccine is not available yet and HCMV therapies result in intolerable side effects and emergence of drug resistant viruses. Discovery of new drugs that inhibit HCMV could significantly reduce morbidity and mortality from this pathogen. Towards this goal we

have developed and characterized a luciferase HCMV reporter that was initially used in 96 well plates and then optimized to 1536-well plates for utilization in screening of the MLSMR collection, the NGCG diversity collection and the NCGC Pharmaceutical Collection of approved and investigational drugs.

Source: NCGC
Grant Number: 1R01DC013550

vi. PSIP1

Description

HIV-1 integrase (IN) is an important therapeutic target in the fight against AIDS as its function is essential for viral replication. Multimeric IN catalyzes pair-wise integration of the linear viral DNA ends in a two-step reaction. The main novelty of the current approach is to discover new probes that stabilize functionally compromised IN multimers rather than interfere with IN multimerization (referred to as MINIs [multimeric IN inhibitors]). LEDGF/p75 is the principal cellular binding partner of HIV-1 IN and is essential for effective integration. Selective LEDGF/p75-IN inhibitors are referred to as LINIs. Using an in vitro model system we have demonstrated that HIV-1 IN is a highly dynamic protein and its ordered, temporal assembly with cognate DNA and cellular co-factor LEDGF/p75 is essential for catalytic activities. To form the stable and functional IN-viral DNA complex, individual IN subunits need to assemble in the presence of viral DNA. LEDGF/p75 promotes IN multimerization in the absence of viral DNA, however these preformed IN multimers are catalytically inactive. Since LEDGF/p75 is required for effective concerted integration in infected cells, it has been speculated that LEDGF/p75 engages preintegration complexes (PICs) only after the stable IN-viral DNA complex is formed. Our novel primary assay of LEDGF/p75-dependent integration will capture both MINIs and LINIs, whereas the two secondary assays of IN multimerization and IN-LEDGF/p75 binding will delineate these two classes of probes.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: Ohio State University
Assay Provider: Mamuka Kvaratskhelia, Ohio State University
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: R01 AI081581
Grant Proposal PI: Mamuka Kvaratskhelia, Ohio State University
External Assay ID: LEDGF-p75_INH_TRFRET_1536_1X%INH PRUN

vii. vpr

Description

Human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, contains a protein called viral protein R (Vpr) that plays an important role in the viral life cycle and the pathogenesis of HIV-1.

Vpr plays multiple roles during the viral life cycle and displays several distinct activities in host cells. These Vpr specific activities include cytoplasmic-nuclear shuttling, induction of cell cycle G2 arrest and cell killing. The cytoplasmic-nuclear shuttling is believed to participate in nuclear transport of the proviral integration complex (PIC). The cell cycle G2 arrest induced by Vpr is thought to suppress human immune functions by preventing T cell clonal expansion and to provide an optimized cellular environment for maximal levels of viral replication. In addition, Vpr induces apoptosis, which may contribute to the depletion of CD4 T cells in HIV-

infected patients. Since the Vpr-specific activities have been linked to such clinical manifestation of AIDS as activation of viral replication, suppression of host immune responses and depletion of CD4+ T-lymphocytes, identification of new molecular probes that can inhibit the Vpr activities could potentially provide a new approach to reduce Vpr-mediated detrimental effects in HIV-infected patients, and thus prolong patient's lives. Currently, there are no anti-Vpr drugs.
A fission yeast (Schizosaccharomyces pombe) model system for the Vpr studies has been developed which measures HIV-1 Vpr-induced cell death. This assay will be used for to screen the NIH Molecular Libraries Small Molecule Repository (MLSMR) identify small molecule HIV-1 Vpr inhibitors.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: NS063880
Assay Submitter (PI): Richard Zhao, University of Maryland

viii. vif

Description

APOBEC3G (A3G) is a cellular cytidine deaminase that restricts HIV-1 replication by inducing G-to-A hypermutation in viral DNA (PMID: 12809610, Harris 2003; PMID: 12808466, Mangeat) and by deamination-independent mechanisms (PMID: 16912295 , Bishop; PMID: 15668174, Newman) . HIV-1 Vif binds to A3G and induces its proteasomal degradation (PMID: 14672928, Mehle, PMID: 14528300, Sheehy). Therefore this interaction represents a potential therapeutic target. To identify compounds that inhibit the interaction between HIV-1 Vif and A3G in a high-throughput format, a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) assay was developed using LANCE (Lanthanide Chelate Excite) reagents. In this assay, interaction between purified GST-Vif residues 1-94 (1-94 GST-Vif), which includes the A3G binding site (mapped to residues 40-72), and a synthetic biotinylated peptide containing A3G residues 110-148 (bio-A3G), which includes the Vif-binding site, is detected by Europium (Eu- donor fluorophore)-labeled anti-GST antibodies and Streptavidin-Ulight (acceptor fluorophore). Interaction between Vif and A3G brings Eu and Ulight into close proximity (~9 nm or less), which supports energy transfer between these molecules measured as a FRET signal. Thus, attenuation of Vif-A3G interaction is expected to result in FRET signal reduction. A total of 262,345 compounds were screened and the average Z-value calculated was 0.77. Active compounds from the primary screen were selected using the mean of all compound results plus 3x the standard deviation. These compounds were retested using the same assay in a dose response format to assess concentration response.

Marintha L. Heil, P.I., Roger G. Ptak, Co-P.I. (Southern Research Institute, Birmingham, Alabama)
Dana H. Gabuzda (Dana Farber Cancer Institute), Assay Provider
Contract: N01-AI-70042 / HHSN272200700042C "In Vitro Testing Resources for AIDS Therapeutic Development, Part B: Specialized In Vitro Virological Assays for HIV Therapeutics and Topical Microbicides"
Compound Samples from MLSMR

ix. NS1 Protein Function

Description

Influenza is a world-wide public health problem and emerging forms of the virus have the potential to cause a pandemic of equal or greater magnitude to the outbreaks recorded in

1918, 1957 or 1968. Vaccine development is proceeding and there also exist two classes of anti-influenza compounds. However these therapeutic modalities are neither fully effective nor widely enough available to fulfill global needs. In addition their potential usefulness against newly emergent strains is not known. Efforts are needed to develop novel agents against influenza virus, including broad-spectrum agents. Identification of small molecules that inhibit NS1 function either directly or by interfering with specific cellular pathways may be a key to increasing our defense against the virus.

We have miniaturized and optimized a cell based assay in which NS1 from influenza A is expressed in the budding yeast S. cerevisiae. NS1 is a multi-functional protein that counters the host innate immune response and facilitates viral versus cellular gene expression. Expression of NS1 causes a pronounced slow growth phenotype in yeast due to its intrinsic molecular activities. Small molecules that suppress the slow growth phenotype can be identified by a straightforward growth recovery assay using optical density (OD) as the measurement. The same yeast strain not expressing NS1 was used as positive control in the yeast growth recovery assay.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH084878-01A1
Assay Submitter (PI): Daniel Engel

x. NS1 H1N1

Description

Influenza viruses are negative-sense, single-stranded RNA viruses that infect the upper, and occasionally the lower, respiratory tracts In the US. Illness from infections by influenza virus has caused an average of approximately 36,000 deaths from 1990-1999 and 226,000 hospitalizations during 1979-2001 (Thompson, W. W., et al. PMID: 15367555), (Thompson, W. W., et al. PMID:12517228). Influenza A viruses (IAV), which also infect a wide number of avian and mammalian species, pose a considerable public health burden with epidemic and pandemic potential. This is particularly evident with the new strain of "swine flu virus" which emerged recently in Mexico and spread to different parts of the North America (MMWR Morb Mortal Wkly Rep PMID:19390508). The most devastating IAV pandemic occurred in 1918 which resulted in approximately 30 million deaths worldwide (Reid, A. H., et al. PMID: 11226857).

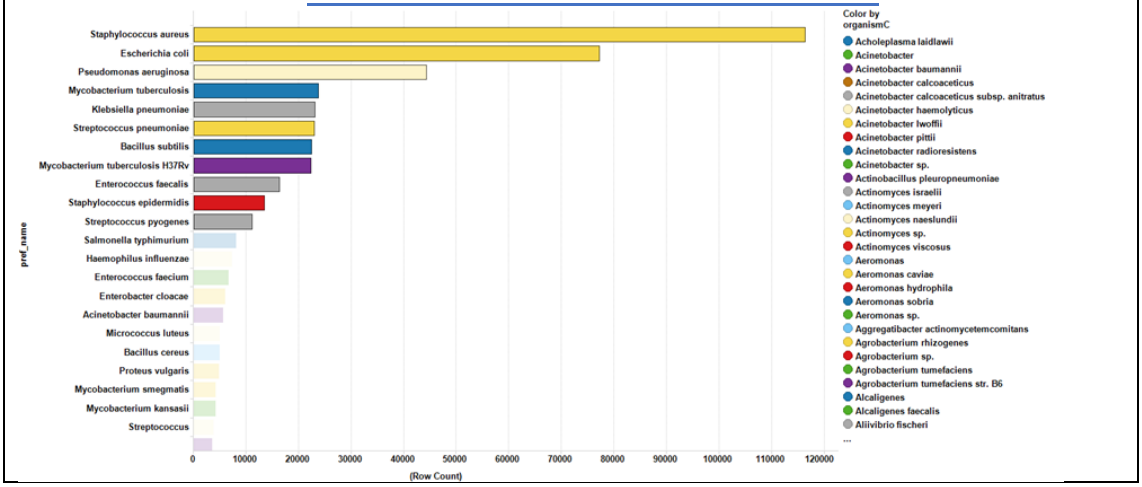
Antivirals are widely used in treatment of epidemic and pandemic IAV infections. Certainly, the lack of availability of vaccines early in IAV pandemics has driven the search for effective antivirals. One potential and largely unexplored IAV target is to disarm viral proteins that modulate the host's antiviral response. NS1A protein is a critical viral protein in this regard. During viral infection, infected cells of the host mount a potent and diverse antiviral defense response to prevent virus proliferation (Randall, R. E., et al. PMID: 18089727). To perpetuate, IAV have evolved multiple mechanisms to circumvent these host defense mechanisms. Strategies include those which are strain-specific such as increased replication speed (Grimm, D., et al. PMID: 17426143), (Kurokawa, M., et al. PMID: 10202186) or those that reduce sensitivity to host-cell antiviral defense mechanisms (Dittmann, J., et al. PMID: 17970694). The NS1A protein inhibits the interferon-alpha-beta-induced 2'-5'-oligo synthetase. Drugs that target the NS1A double-stranded (ds) RNA binding domain would inhibit IAV replication.

The N terminus of the NS1A protein (NS1A) of IAV is a highly conserved, multifunctional viral protein which interacts with host dsRNA (Cheng, A., et al. PMID: 18813227). We have developed an AlphaScreen (Perkin Elmer)-based 1536-well high throughput screen (HTS) for NS1A to identify small molecule inhibitors of the dsRNA binding activity. Identification of probes for this activity would provide valuable tools for probing its dsRNA binding function within the cell. Further, this domain could also serve as a target for antiviral drug discovery. To further validate the assay we developed for NS1A dsRNA binding activity, we screened a small library (Prestwick) of 1120 compounds using HTS and identified several compounds that were reported to have nucleic acid binding properties.

Source: Southern Research Specialized Biocontainment Screening Center
Grant Number: 1 R03 MH089536-01

b. Antibacterial

i. Cell death on 11 Antibacterial strains



c. Antifungal

i. fluconazole-resistant C.albicans

Description

The basic assay strategy will consist of fluconazole-resistant C. albicans clinical isolate cultured in 1536-well format in the presence of a sub-toxic concentration of fluconazole. Test compounds that inhibit subsequent growth in the presence of fluconazole will merit further evaluation for their synergy with fluconazole. This whole cell phenotypic screening approach will only capture compounds that retain activity in biological media and are capable of entering and accumulating in fungi to bioactive concentrations. Grossly cytotoxic compounds will be removed through subsequent counterscreens. Compound activity will be measured by the metabolism of Alamar Blue, a cell stain that is metabolized to a fluorescent product by living cells but that remains non-fluorescent in wells with growth-inhibited organisms.

Probe attributes:
a. Compounds that inhibit yeast growth in the presence, but not in the absence of 8 ug/ ml fluconazole.
b. Compounds that show a 10-fold specificity between the primary Candida test strain and mammalian cells.

c. Compounds that exhibit 10X greater inhibition against fungus than the hsp90 chaperone and /or calcineurin.
d. IC50 < 1uM

Expected Outcome: Active wells will show a reduced fluorescence intensity due to a reduction in the amount of Alamar Blue dye metabolized by fewer viable microorganisms.

Broad Institute: Reversing Antifungal Drug Resistance
Project ID: 2037
Primary Collaborators: Susan Lindquist, Whitehead Institute for Biomedical Research, sll@wi.mit.edu

ii. antifungal efflux pumps inhibitors

Description

Fungal infections, exemplified by oral and invasive candidiasis, cause considerable morbidity and mortality in the immunocompromised. Treatment of patients with fungal infections is severely hampered by the development of antifungal drug resistance [Cannon, et al 2009]. The goal of this project is to address this health need by discovering novel chemical compounds that reverse antifungal drug resistance by inhibiting the drug efflux pump molecules in the fungal cell membrane, overexpression of which is the major cause of resistance in clinical isolates of most Candida species [Niimi, et al. 2004].

Utilizing a a panel of yeast strains expressing efflux pumps from the following clinically relevant fungi: Candida albicans, Candida glabrata, and Candida krusei [Lamping and Cannon, 2010], this approach analyzes three 'sentinel' strains in a phenotypic HTS which measures efflux of the fluorescent pump substrate Nile Red. Follow-up analysis on confirmed HTS hits will be conducted in single-plex assays using each strain individually to identify the target detected in the primary HTS.

Assay Support: 1 R03 MH087406-01A1
Project Title: Identification of broad-spectrum antifungal efflux pump inhibitors
PI: Richard Cannon
Screening Center PI: Larry Sklar
Chemistry Center PI: Craig Lindsley
Assay Implementation: J Jacob Strouse, Susan Young, Stephanie Chavez, Dominique Perez, Matthew Garcia, Travis Houston, Keon Ahghar, Terry Foutz, Anna Waller, Annette Evangelisti, Mark Carter, Virginia Salas

iii. VMA11

Description

Distributed among the endomembrane system of all eukaryotic cells, V-ATPase proton pumps are responsible for acidification of intracellular compartments. V-ATPases maintain the low pH necessary for endocytic and exocytic vesicular transport, zymogen activation, and protein sorting and degradation. Enveloped viruses, such as influenza virus, as well as toxins, such as diphtheria toxin, enter cells via acidic endosomal compartments, in which low pH is maintained by V-ATPases. Because the pH influences most aspects of cell physiology most cells will die without functional V-ATPases. Inhibition of the V-ATPase is a quick-acting way to disrupt vital cell functions. It is probably for this reason that some organisms evolved to produce natural products that inhibit V-ATPases. However, these inhibitors have poor selectivity and/or potency. For instance, bafilomycins and concanamycins, which are inhibitors broadly used to study V-ATPases in vitro and in vivo

cannot discriminate between fungal and mammalian V-ATPases; other compounds preferentially inhibit the mammalian V-ATPase (salicylihalamides and lobatamides) but cannot discriminate between human V-ATPase tissue specific isoforms; and those compounds that preferentially inhibit fungal V-ATPases (chondropsines) have very low potency, even lower than bafilomicyns and concanamycins which require micromolar concentrations to inhibit the yeast pump in vivo. This phenotypic study is designed to identify small molecules that inhibit fungal V-ATPase pumps with high potency in vivo.

University of New Mexico
Assay Support: 1 R03 DA031666-01A1
Project Title: Flow Cytometry HTS of Small Molecules that Regulate V-ATPase Proton Transport in Yeast
Assay Provider: Karlett Parra Ph.D
Lead Biologist: Catherine Prudom Ph.D., Mark Carter MS
Assay Implementation: Travis Houston, Keon Ahghar, Stephanie Chavez, Dominique Perez, Matthew Garcia, Sahba Charkhzarrin, Anna Waller Ph.D, Annette Evangelisti Ph.D

iv. tRNA 2'-phosphotransferase

Description

The process of transcription converts the DNA sequences found in genes into mRNA. This process is coupled to the subsequent removal of mRNA introns by splicing, which additionally serves to increase proteome complexity. Intron splicing also occurs for transfer RNA (tRNA), which functions in the delivery of amino acids to the growing peptide chain as directed by the associated triplet codon during translation. This essential splicing step removes an intron in the anticodon loop, and occurs in organisms from Archaea to Eukarya. In yeast the final pre-tRNA splicing step is catalyzed by 2'-phosphotransferase 1 (Tpt1), which transfers the 2'-phosphate from pre-tRNA to NAD to produce the mature tRNA. Although early studies suggested that Tpt1 was essential for yeast and humans, a recent report showing that null mice mutants of Trpt1 [the mammalian homologue of Tpt1] are normal and fertile, with no detectable defects in tRNA splicing or non-conventional splicing, suggests that the gene is dispensable in mammals. As a result, the identification of selective inhibitors of Tpt1 may prove useful in the elucidation of the specific splicing function of this enzyme in diverse organisms and as potential anti-fungal agents.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: Heather Harding, New York University School of Medicine
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1-R03-DA026554-01
Grant Proposal PI: Heather Harding, New York University School of Medicine
External Assay ID: TPT1_INH_FP_1536_1X%INH

d. Protozoan

i. T. Cruzi replication

Description

The assay detects intracellular trypanosomes that are replicating inside host cells. NIH3T3 cells are trypsinized, counted, and plated in 384-well tissue culture plates. After plating the NIH3T3 cells, compounds are added to the wells. T. cruzi that are genetically modified to

express b-galactosidase are then harvested and active Trypomastigotes form are counted and co-cultured with the NIH3T3 cells. After 90 hours of co-culture, all cells in the well are lysed and b-galactosidase production is detected using a luminescent reporter system (GalScreen).

Expected Outcome: Compounds significantly suppressing luminescence, and therefore b-galactosidases expression will be identified as hits in the screen. Compounds that inhibit luminescence activity may kill T. cruzi, inhibit T. cruzi invasion or inhibit development of the parasite within the host cell. Compounds that are toxic to the host cell will be excluded in secondary assays.

Source: Broad Institute
Grant Number: MH085673-01
Primary Collaborators: Ana Rodriguez, New York University, Ana.Rodriguez@nyumc.org; Esther Bettiol,Merck/Serano, estherbettiol@hotmail.com

ii. Leishmania promastigote

Description

Infection with Leishmania represents a major health concern in the developing world, with approximately 1.2 to 1.5 million cases reported annually and 350 million people (globally) at risk of infection. The limited number of available leishmaniasis treatments is complicated by (1) serious (toxic) side effects; and (2) an increase in chemoresistance. Therefore, the identification of new small molecules for the treatment of leishmaniasis is a critical. A simple, inexpensive and HTS amenable methodology (Alamar blue) has been implemented to measure Leishmania spp. promastigote drug susceptibility. Alamar blue has been successfully used as a screening format for Leishmania promastigotes. Alamar blue is an oxidation-reduction indicator which is not toxic for cells (or in this case, promastigotes) even over long incubation times, which is reduced, and changes color from blue to red in living cells (or promastigotes). A colorimetric or fluorometric (560Ex/590Em) reading is obtained and correlates with Leishmania promastigote number. Thus, promastigote drug susceptibility can be determined. Described here are HTS data that were collected upon implementation of the assay. The assay was developed and evaluated according to the PMLSC/UPDDI assay development and implementation guidelines.

Source: University of Pittsburgh Molecular Library Screening Center
Assay Provider: John Lazo, University of Virginia
Assay Performer: Elizabeth Sharlow, University of Virginia
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 5 P50 GM069663
Grant Proposal PI: John Lazo, University of Virginia

iii. Plasmodium delayed death (apicoplast)

Description

The goal of this screen is to discover new antimalarial compounds that act by inhibiting the development of the apicoplast in the malarial parasite Plasmodium falciparum. The biochemical processes that make this organelle essential for erythrocytic stage parasites are not well understood. However, antibiotics such as azithromycin and tetracycline, which target the apicoplast translational machinery, have a potent antimalarial effect. The killing caused by these drugs is unusual in that it does not appear to affect the first generation of parasites that are exposed to the drug, but rather manifests itself in their progeny.

We have developed a cell-based assay that measures parasite growth based on the expression of an integrated copy of a firefly luciferase reporter. To detect small molecules that cause this "delayed death" phenotype, erythrocytes infected with the luciferase-expressing parasites were incubated with compounds for either one or two generations, corresponding to 48 and 96 hours, respectively. Compounds that inhibit parasite growth in the second generation, but not the first, should be enriched in antimalarials that target the apicoplast. Growth inhibition is detected by a decrease in luciferase activity.

MLPCN Grant: R21 NS059500
Assay Provider: David Fidock and Eric Ekland, Columbia University

iv. Plasmodium cell death

Description

The goal of this screen is to discover new antimalarial compounds that act by inhibiting the development of the apicoplast in the malarial parasite Plasmodium falciparum. The biochemical processes that make this organelle essential for erythrocytic stage parasites are not well understood. However, antibiotics such as azithromycin and tetracycline, which target the apicoplast translational machinery, have a potent antimalarial effect. The killing caused by these drugs is unusual in that it does not appear to affect the first generation of parasites that are exposed to the drug, but rather manifests itself in their progeny.

We have developed a cell-based assay that measures parasite growth based on the expression of an integrated copy of a firefly luciferase reporter. To detect small molecules that cause this "delayed death" phenotype, erythrocytes infected with the luciferase-expressing parasites were incubated with compounds for either one or two generations, corresponding to 48 and 96 hours, respectively. Compounds that inhibit parasite growth in the second generation, but not the first, should be enriched in antimalarials that target the apicoplast. Growth inhibition is detected by a decrease in luciferase activity.

MLPCN Grant: R21 NS059500
Assay Provider: David Fidock and Eric Ekland, Columbia University

v. P. falciparum 3d7

Description

This assay uses levels of P. falciparum lactate dehydrogenase as surrogate of parasite growth. Inhibition of 3D7 growth by compounds has been determined in this assay.

Source: GlaxoSmithKline (GSK)

vi. P. falciparum Dd2

Description

This assay uses levels of P. falciparum lactate dehydrogenase as surrogate of parasite growth. Inhibition of Dd2 growth by compounds has been determined in this assay.

Source: GlaxoSmithKline (GSK)

vii. P. falciparum erythrocyte W2

Description

The growing resistance to current first-line antimalarial drugs represents a major health challenge. To facilitate the discovery of new antimalarials, we have implemented an efficient

and robust high-throughput cell-based screen (1,536-well format) based on proliferation of Plasmodium falciparum (Pf) in erythrocytes. From a screen of approximately 1.7 million compounds, we identified a diverse collection of approximately 6,000 small molecules comprised of >530 distinct scaffolds, all of which show potent antimalarial activity (<1.25 microM). Most known antimalarials were identified in this screen, thus validating our approach. In addition, we identified many novel chemical scaffolds, which likely act through both known and novel pathways. We further show that in some cases the mechanism of action of these antimalarials can be determined by in silico compound activity profiling. This method uses large datasets from unrelated cellular and biochemical screens and the guilt-by-association principle to predict which cellular pathway and/or protein target is being inhibited by select compounds. In addition, the screening method has the potential to provide the malaria community with many new starting points for the development of biological probes and drugs with novel antiparasitic activities.

Source: Novartis
Proc Natl Acad Sci U S A. 2008 Jul 1;105(26):9059-64. doi: 10.1073/pnas.0802982105. Epub 2008 Jun 25.
In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen.
Plouffe D, Brinker A, McNamara C, Henson K, Kato N, Kuhen K, Nagle A, Adrián F, Matzen JT, Anderson P, Nam TG, Gray NS, Chatterjee A, Janes J, Yan SF, Trager R, Caldwell JS, Schultz PG, Zhou Y, Winzeler EA.
Genomics Institute of the Novartis Research Foundation, San Diego, CA 92121, USA.

viii. P. Falciparum erythrocyte 3D7

Description

The growing resistance to current first-line antimalarial drugs represents a major health challenge. To facilitate the discovery of new antimalarials, we have implemented an efficient and robust high-throughput cell-based screen (1,536-well format) based on proliferation of Plasmodium falciparum (Pf) in erythrocytes. From a screen of approximately 1.7 million compounds, we identified a diverse collection of approximately 6,000 small molecules comprised of >530 distinct scaffolds, all of which show potent antimalarial activity (<1.25 microM). Most known antimalarials were identified in this screen, thus validating our approach. In addition, we identified many novel chemical scaffolds, which likely act through both known and novel pathways. We further show that in some cases the mechanism of action of these antimalarials can be determined by in silico compound activity profiling. This method uses large datasets from unrelated cellular and biochemical screens and the guilt-by-association principle to predict which cellular pathway and/or protein target is being inhibited by select compounds. In addition, the screening method has the potential to provide the malaria community with many new starting points for the development of biological probes and drugs with novel antiparasitic activities.

Source: Novartis
Proc Natl Acad Sci U S A. 2008 Jul 1;105(26):9059-64. doi: 10.1073/pnas.0802982105. Epub 2008 Jun 25.
In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen.
Plouffe D, Brinker A, McNamara C, Henson K, Kato N, Kuhen K, Nagle A, Adrián F, Matzen JT, Anderson P, Nam TG, Gray NS, Chatterjee A, Janes J, Yan SF, Trager R, Caldwell JS, Schultz PG, Zhou Y, Winzeler EA.
Genomics Institute of the Novartis Research Foundation, San Diego, CA 92121, USA.

ix. met-tRNA synt

Description

Human African trypanosomiasis (HAT; also called sleeping sickness) is a neglected tropical disease that is caused by the protozoan Trypanosoma brucei, which employs the tsetse fly as its insect vector. Related tropical diseases include Chagas disease (caused by Trypanosoma cruzi) and leishmaniasis (caused by Leishmania species). Each of these diseases has a major impact on human health around the world and they lack adequate chemotherapeutic treatment options (1), as current therapies suffer from poor efficacy, oral bioavailability (2), toxicity, and difficult treatment regimens (3). As a result there is a great need to develop novel, more selective, and effective treatments (4). The aminoacyl-tRNA synthetases (aaRS) play essential roles in protein synthesis and cell survival and thus are attractive targets for the design of novel chemotherapeutic agents for these diseases (3). aaRS enzymes are essential to translating nucleotide-encoded gene sequences into proteins. Thus, inhibitors that interfere with these enzymes will inhibit formation of properly charged tRNA, leading to accumulation of uncharged tRNA on the ribosome, and disruption of normal protein chain elongation during translation, which are detrimental to cell viability. In particular, genomic studies have revealed sequence differences between the T. brucei trypanosome and mammalian methionyl-tRNA synthetases (MetRSs: which are members of the aaRS family), suggesting that selective inhibition of this enzyme and protozoan death can be achieved using drug-like molecules (2). Using RNA interference, T. brucei MetRS has been shown to be essential for parasite survival (3). In addition, since the MetRS enzymes from Trypanosomatid organisms are highly homologous (particularly in the methionine-ATP binding pocket) it is possible that compounds active against T. brucei MetRS will exhibit activity against the MetRS enzymes from T. cruzi and Leishmania.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: University of Washington
Assay Provider: Wilhelmus Hol, University of Washington
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R01 AI084004-01A1
Grant Proposal PI: Wilhelmus Hol, University of Washington
External Assay ID: METRS_INH_LUMI_1536_1X%INH PRUN

e. Prion

i. PRNP

Description

Prion diseases are lethal infectious neurodegenerative diseases affecting animals and humans. In humans, Creutzfeldt-Jakob Disease (CJD) is either sporadic, affecting mainly people over 60 years, iatrogenic, or genetic with a high penetrance. Prion diseases are characterized by the accumulation, in brain and lymphoid tissue of PrPsc, a misfolded, aggregated form of the host prion protein PrP. PrPsc is thought to be the main or only constituent of the prion, the infectious agent. PrP is a glycosylphosphatidylinositol (GPI)-anchored protein of 254 amino acids attached to the cell surface in microdomains known as lipid rafts, and tends to aggregate into rod-like structures. The encoded protein contains a highly flexible region of five tandem octapeptide repeats. Mutations in the repeat region as well as elsewhere in this gene have been associated with Creutzfeldt-Jakob disease, fatal familial insomnia, and Gerstmann-Straussler disease.

Prion replication is based on the structural conversion of the host prion protein into its harmful misfolded counterpart. In the sporadic form of CJD, the initial misfolding of PrP may be a rare, stochastic event. In familial CJD, this event is triggered by certain mutations in the protein. When the disease is transmitted, PrPsc acts as a template and "seeds" the misfolding of the host PrP. Prion diseases are not curable and the survival time is typically 6 to 12 months after the onset of symptoms. Bovine spongiform encephalopathy (BSE), or mad cow disease, is transmissible to humans. While about 200 individuals are known to have succumbed to "human BSE" (variant CJD), the number of infected but as yet asymptomatic people is unknown because there is no preclinical diagnostic test for prion diseases and the incubation time may extend to decades; this is of particular concern because the disease is transmissible by blood transfusion. As a result, the identification of compounds that suppress cell surface PrP and thereby suppress prion replication may provide useful tools for the therapy of prion diseases, and for study of PrP biosynthetic and cellular trafficking pathways.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Corinne Lasmezas, The Scripps Research Institute Molecular Screening Center
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R03 DA035192-01
Grant Proposal PI: Corinne Lasmezas, The Scripps Research Institute Molecular Screening Center
External Assay ID: PRPC_INH_TRFRET_1536_1X%INH PRUN

5. Metabolism

a. Energy balance, obesity

i. Orexin 1

Description

Heterotrimeric G-protein coupled receptors (GPCRs) are major targets for disease therapeutics, due in part to their broad tissue distribution, structural diversity, varied modes of action, and disease association. Most non-sensory GPCRs are expressed in the brain and regulate critical neuronal functions involved in feeding, sleep, mood, and addiction. For example, in the lateral hypothalamic region of the brain, two orexin neuropeptides (orexin A and orexin B) derived from proteolytic processing of the same orexin precursor, signal through the Gq-coupled GPCRs OX1R and OX2R to stimulate food consumption. OX1R binds orexin A selectively, while OX2R binds both orexin A and orexin B. Recently, signaling by orexin A through OX1R has been shown to play a critical role in cocaine-seeking behavior and morphine withdrawal. Additional studies reveal OX1R involvement in behavioral plasticity, the sleep-wake cycle, and gastric acid secretion, and that OX1R may bind other neuropeptides such as neuropeptide Y and secretin. As a result, the identification of a selective OX1R antagonist would serve as a useful tool for exploring orexin biology, and the role of OX1R in drug addiction.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Patricia McDonald, TSRI
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: R01 DA023915-02
Grant Proposal PI: Patricia McDonald
External Assay ID: OX1R_ANT_FRET_1536_1X%INH PRUN IP-ONE

ii. MC4R

Description

Heterotrimeric G-protein coupled receptors (GPCRs) are major targets for disease therapeutics, due in part to their broad tissue distribution, structural diversity, varied modes of action, and disease-associated mutations. However, it has recently been demonstrated that GPCRs do not only signal in this simplistic fashion, but rather activate a network of downstream effects comprised of parallel signal transduction pathways. GPCR ligands biased towards induction or blockade of specific signaling pathways may have different physiology compared with unbiased molecules, through selective engagement of a desired subset of signal cascades. For example, the melanocortin 4 receptor (MC4R) transduces its signal via coupling to Gs and adenylyl cyclase activation, and is involved in the regulation of energy homeostasis and chronic disease-associated cachexia. Recent studies indicate that classical antagonists do not mimic MC4R regulation by its endogenous ligand, Agouti-Related Protein (AgRP). Indeed, AgRP has several actions including antagonizing Gs-mediated adenylyl cyclase activation, inducing beta-arrestin recruitment and MC4R endocytosis, as well as stimulating Gi-mediated inhibition of adenylyl cyclase. As a result, the identification of small molecules that act as biased MC4R ligands, by blocking Gs protein coupling but stimulating beta-arrestin functions and/or Gi protein coupling, may lead to a better understanding of this receptor and its role in metabolic/wasting diseases.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRISMC)
Center Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Scott DeWire, Trevena Inc
Network: Molecular Library Probe Production Center Network (MLPCN)
Grant Proposal Number: 1 RC2 MH090877-01
Grant Proposal PI: Scott DeWire, Trevena Inc
External Assay ID: MC4R_AG_LUMI_1536_1X%ACT PRUN

iii. Rev-erb Alpha

Description

Rev-Erbalpha is a member of the nuclear receptor (NR) superfamily. The Rev-Erbs regulate gene programs related to fat oxidation, cholesterol metabolism, glycolysis and triglyceride synthesis. These receptors also control the expression of circadian core clock components in peripheral tissues, as well as the central circadian oscillator in the hypothalamus. We previously identified heme as the physiological ligand for both of these receptors, and demonstrated that reversible ligand binding leads to the recruitment of the corepressor NCoR, and target gene repression. Our laboratory also determined the crystal structure of the DNA-binding domain of Rev-Erbalpha bound to DNA, showing how these receptors recognize their response elements. A single synthetic class of Rev-Erbalpha/beta dual modulator was recently developed and shown to increase energy expenditure, decrease fat mass, and lower plasma triglyceride, cholesterol and glucose levels in animals on a high-fat

diet. However, these molecules also crossed the blood-brain barrier to influence circadian behavior by modulating Rev-Erb beta actions within the hypothalamus.

Based on those important findings, we have developed an innovative screening approach to discover new classes of Rev-Erb modulators, including molecules with more selective actions on Rev-Erbalpha receptors. Molecules that don't cross the blood-brain barrier are also desired, so as to capture the metabolic benefits in peripheral tissues without impacting the hypothalamic circadian clock. Our primary screening assay, counter-assay, and entire battery of secondary and tertiary studies have all been optimized at this time. We propose to screen the full MLPCN library and carry out hit-to-lead studies with long-term goal of finding new Rev-Erb modulators that can be developed for treating obesity, diabetes and hyperlipidemia.

Source: Burnham Center for Chemical Genomics
Grant Number: 5R01DK09475

b. insulin sensitization

i. NR2F2

Description

Steroid receptor chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), an orphan nuclear receptor and member of the nuclear receptor superfamily, has been shown to be a critical transcriptional regulator in many different cancer types by promoting angiogenesis, cell proliferation and metastasis. COUP-TFII has widespread tissue distribution in human; detectable expression has been found in every tissue type examined. Currently, the treatment for tumor angiogenesis focuses mainly on blocking VEGFR-2 signaling and has not been effective due to limited efficacy, eventually leading to resistance and/or relapse. COUP-TFII has been shown to promote tumor angiogenesis through modulating multiple angiogenic signals (VEGF/VEGFR-2, Angiopoietin 1/Tie2 and E2F-1) in many different types of cancer. In addition, COUP-TFII is overexpressed in prostate and several other cancers and is an excellent prognostic marker. By including COUP-TFII data with Cyclin D1, p21, PTEN, and Smad4 data in the prognosis, the prognostic accuracy is improved. The expression level of COUP-TFII and its role in regulating tumor growth and metastasis in prostate cancer has been examined, and these data indicate that COUP-TFII positively promotes prostate tumor growth and metastasis. These results provide the rational basis to posit that inhibition of COUP-TFII may offer a novel and broadly efficacious approach for anticancer intervention.

COUP-TFII has also been shown to regulate energy storage and expenditure. We have found that COUP-TFII heterozygous mice have increased mitochondrial biogenesis in white adipose tissue, which results in higher energy expenditure, resulting in resistance to high fat diet-induced obesity and improved glucose homeostasis due to increased insulin sensitivity at peripheral tissues. These results indicate that COUP-TFII has an important role in regulating adipocyte differentiation and energy metabolism. Therefore, COUP-TFII inhibitors could potentially serve as agents to improve insulin sensitivity, enhance energy metabolism, and decrease high fat diet-induced obesity.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Ming-Jer Tsai, Baylor College of Medicine
Network: Molecular Library Probe Production Centers Network (MLPCN)

Grant Proposal Number: R01DK45641
Grant Proposal PI: Ming-Jer Tsai, Baylor College of Medicine
External Assay ID: COUPTFII_INH_LUMI_1536_1X%INH PRUN

ii. RXRA

Description

The Retinoid X receptor (RXR), a nuclear hormone receptor expressed in liver, spleen, placenta, epidermis, central nervous system. These receptors are able to form homo and heterodimers with other nuclear receptors and due to this heterodimerizing ability, RXR functions as master regulators producing diverse physiological effects. Three subtypes of RXR exist; alpha, beta and gamma. Retinoid X receptor alpha (RXR-alpha) has potential roles in metabolic signaling pathways, skin alopecia, dermal cysts, cardiac development, insulin sensitization etc. Therefore screening environmental chemicals on RXR-alpha signaling can be used for the studies focused on such therapeutic applications. For quantification of RXR-alpha ligands signaling, "GeneBLAzer RXR alpha-UAS-bla HEK 293T" (Life Technologies, Carlsbad, CA) cells containing a beta-lactamase reporter-gene under control of Retinoid X receptor-alpha response element were used to screen Tox21 10K library. The cytotoxicity of the Tox21 compound library against the RXR-alpha HEK 293T cell line was tested in parallel by measuring cell viability using CellTiter-Glo assay (Promega Corporation, Madison, WI) in the same wells. The compounds were also tested for auto fluorescence that may interfere with the biological target readout resulting in potential false positives and/or negatives.

National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

c. obesity, diabetes, and cardiovascular disease

i. ABHD5

Description

Adipocytes are important regulators of vertebrate energy stores, in part through the storage of dietary fat (triglyceride) that is mobilized via lipolysis during fasting states for use by tissues such as heart and skeletal muscle. However, in chronic conditions of overnutrition, such as obesity and lipid storage disorders, excess intracellular lipid accumulation and reduced lipolysis leads to cellular lipotoxicity, which contributes to diabetes, atherosclerosis, and cardiomyopathy. The metabolism of cellular lipid is regulated in part by protein-protein interactions near the surface of intracellular lipid droplets. In adipocytes lipolysis is inhibited by the interaction of a protein called abhydrolase domain-containing 5 (ABHD5) with the lipid droplet scaffold protein perilipin A (PLIN). In cells that do not express PLIN, such as myocytes, lipolysis is blocked by similar interactions of ABHD5 with myocyte lipid droplet protein (MLDP). Studies showing reduced lipotoxicity following Plin overexpression, combined with population studies identifying ABHD5 mutations as a cause of the lipid storage disease Chanarin-Dorfman syndrome, suggest that activating lipolysis by blocking interactions of ABHD5 with PLIN or MLDP will increase lipid clearance from adipocytes and other cells, thereby reducing lipotoxicity. As a result, compounds that inhibit these protein interactions may have therapeutic potential for lipid disorders such as obesity, diabetes, and cardiovascular disease.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: James Granneman, Wayne State University
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R21 NS061634-01
Grant Proposal PI: James Granneman, Wayne State University
External Assay ID: ABHD5-MLDP_INH_LUMI_1536_1X%INH PRUN

d. T1Diabetes

i. b cell apoptosis

Description

Type 1 diabetes is caused by autoimmune destruction of insulin-producing beta cells in the pancreas. Human beta-cell apoptosis in this process involves a complex set of signaling cascades initiated by interleukin-1b (IL-1b), interferon-g (IFN-g), and tumor necrosis factor-a (TNF-a). IL-1b and TNF-a induce NFkB expression, while downstream activation of gene expression is thought to occur through nitric oxide (NO) signaling, which both increases the endoplasmic reticulum stress-response pathway and decreases beta-cell function. These effects of cytokines are beta cell-specific, and we aim to find small-molecule suppressors that would have little to no effect on other cell types in the pancreas.

Small molecules that increase beta-cell survival in the presence of cytokines could be of potential clinical benefit to early-stage type 1 diabetic patients. A number of studies have described small molecules with protective effects in the presence of cytokines; most of these were discovered because of their antioxidant or anti-inflammatory effects. Further, small-molecule inhibition of histone deacetylases (HDAC) with suberoylanilide hydroxamic acid (SAHA) or trichostatin A (TSA) can prevent cytokine-induced beta-cell death, presumably by decreasing NFkB transactivation. Therefore, multiple mechanisms may serve to protect beta cells from cytokine-induced apoptosis.

INS1E rat insulinoma cells are similar to pancreatic beta cells because they secrete insulin in response to glucose stimulation. In the primary assay, INS1E cells are treated with 3 cytokines (Interferon-gamma, Tumor necrosis factor-alpha and Interleukin 1-beta) and compounds for 48 hours. The combination of cytokines leads to apoptotic cell death. The level of cell death is inferred my measuring overall ATP levels with Promega's Cell Titer Glo.

Expected Outcome:
Cytokine induced apoptosis leads to cell death and thus, reduced ATP levels. Candidate compounds will prevent cell death and keep ATP levels elevated. ATP is measured with Cell Titer go (Promega) and so increased values are expected. A compound is scored as a hit when both replicates score as 74% activity or higher and has a minimum Z score of 3.

Source: Broad Institute
Grant Number: DP2 DK083048

Primary Collaborators: Bridget Wagner,Broad Institute,Cambridge, MA,bwagner@broadinstitute.org,617-714-7363
Danny Chou,Broad Institute,Cambridge, MA,dchou@broadinstitute.org

e. T2Diabetes

i. Glucose-dependent insulin secretion

Description

Our proposed study seeks to establish a "first-in-class" probe set to target regulators of glucose-dependent insulin secretion. We will use a novel high-throughput luminescent insulin secretion assay to identify compounds that amplify insulin secretion only in the presence of a permissive glucose environment, and then use follow-up assays to identify the signalling pathways affected by these compounds within the beta cell. As a defect in glucose-dependent insulin secretion drives the pathogenesis of type 2 diabetes, small-molecule modulators of this pathway would represent valuable chemical tools and potential leads for the development of therapeutic agents.

Insulin is normally secreted from the pancreatic beta cell in a highly regulated manner subsequent to the
metabolism of glucose and other fuels. Observational studies of patients with impaired glucose tolerance or
overt type 2 diabetes have highlighted that defective insulin secretion is a key predisposing factor for disease. More recently, human genetics has confirmed an etiologic role of inherited variants in the pathogenesis of diabetes, many of which impart risk by decreasing insulin secretion. Unfortunately, the responsible molecular mechanisms remain obscure, largely because we have few tools for exploring glucose-dependent insulin secretion in the beta cell. Moreover, all current FDA-approved small molecule therapeutics that increase insulin secretion do so irrespective of glucose concentration, limiting their utility and patient compliance due to the associated risk of severe hypoglycemia. These drugs may also contribute to so-called ###beta-cell burnout### as a result of their chronic stimulation on the cells secretory machinery, even under low glucose conditions. The assay uses a rat Beta Cell line (INS-1E) engineered to contain a Gaussia luciferase reporter. Beta-cell lines expressing this construct co-secrete luciferase and insulin in close correlation.

Expected Outcome: Compounds that cause an increase in insulin secretion result in an increase in luminescence. Active hits are determined as wells with luminesncent signal four standard deviations above the per-plate population of neutral control wells (approximately 40 % of the IBMX positive control.)

Primary Collaborators: David Altshuler,Broad Institute,altshul@broadinstitute.org,617-714-7030. Sean Burns,Broad Institute, sburns@broadinstitute.org, 617-714-7885
Source: Broad Institute
Grant Number: 1 R03 DA035188-01

6. Neurodegeneration

a. AD

i. NFKB1

Description

The pharmacological treatment of neurodegenerative disorders has been a disappointment when compared to the successes obtained in stroke, other neurological diseases like seizures, and in mental health diseases. It has to be said that the pathogenesis of neurodegenerative disorders and their early diagnosis represent a definite obstacle to effective intervention.

Nuclear factor kB (NF-kB) is a key cellular signalling factor in the central nervous system. Although NF-kB signalling pathways have been extensively investigated in cancer and in immunological diseases, NF-kB role in the central nervous system physiology and pathology in non inflammatory disorders of the brain is still unclear. NF-kB has an important role as an inhibitor of neuronal apoptosis and at least in this capacity it represents an interesting target to pursue. Recent evidence has also pointed out that this signalling system is involved in the resilience of neurons and in their ability to survive disparate insults. Functional knock out models of NF-kB, achieved via over-expression of its natural repressor I-kB, have shown that neurons from these animals are more sensitive to insults including trauma, Beta-amyloid toxicity and excitotoxicity. Also a direct neuroprotective effect from both non apoptotic and apoptotic models of neurodegeneration has been attributed to direct or indirect activation of NF-kB signaling. In addition, to the direct neuroprotective effect NF-kB up-regulation could also be of value in improving learning and memory. In fact, NF-kB signalling has been shown to improve long term potentiation and long term depression, two models of learning at cellular level. This background is of great appeal because up regulation of NF-kB in neurons could be useful in attacking, at the same time, neuronal degeneration and deterioration of neuronal functions associated with loss of learning and memory as seen in Alzheimer's disease. To pursue this scope, we have set up a novel human cell-based assay to identify small molecules able to up-regulate NF-kB expression for high throughput screening.

The cell line developed for this assay, which is used to identify pharmacological probes for the activation of NF-kB, was prepared as follows. The SH-5YSY human neuroblastoma cell line was doubly stably transfected with a reporter construct coding the human NF-kB promoter driving the expression of the firefly luciferase and a reporter coding the resistance to the antibiotic blasticidin. TNF-alpha was used as a positive control as it a potent enhancer of NF-kB expression. Negative control wells were treated with vehicle only.

Southern Research Molecular Libraries Screening Center (SRMLSC)
Southern Research Institute (Birmingham, Alabama)
NIH Molecular Libraries Screening Centers Network (MLSCN)
Submitted by Dr. Maurizio Grimaldi (Neuropharmacology Laboratory, Southern Research Institute)
Award: R03 MH082367-01

ii. APP

Description

Novel reagents that inhibit the Amyloid Precursor Protein (APP) translation and subsequently the Amyloid beta protein production, that is elevated in Alzheimer's disease, could lead to the discovery of therapeutic drugs for the disease. MLSMR 160K library was screened for small molecules that suppress the Amyloid Precursor Protein (APP) translation

by binding to the 5'Untranslated Region of the APP mRNA. It utilizes a stable neuroblastoma SH-SY5Y transfectants that express Luciferase under the translational control of 146 nucleotides from the 5'UTR of APP mRNA. It had been shown that this region forms a stem loop which is, in turn, a significant regulator of the APP levels in the brain (1, 2). The effects of compounds addition on APP levels is measured by quantification of luciferase expression levels with a bioluminescence assay system. The assay was previously utilized by Jack Rogers for HTS screen of a 110,000 compounds library from the Laboratory for Drug Discovery on Neurodegeneration (LDDN)(3).

Data Source: Columbia University Molecular Screening Center
Source (MLSCN Center Name): Columbia University Molecular Screening Center
Center Affiliation: Columbia University Molecular Screening Center
Assay Provider: Jack T. Rogers Genetics and Ageing Research Unit; Psychiatry Department, Massachusetts General Hospital, Boston.
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number: MH079854-01

iii. IDE

Description

Alzheimer's disease (AD) is characterized by accumulation of amyloid beta-protein (A-beta; Abeta) in brain regions involved in memory and cognition. The steady-state levels of AB reflect a balance between its production via beta- and gamma-secretases and its catabolism by proteolytic degradation. Because Abeta cleavage products are less neurotoxic than intact Abeta, enzymes that cleave Abeta are of therapeutic interest for AD. In fact, upregulation of Abeta-degrading proteases can prevent AD-like pathology in beta-amyloid precursor protein (APP) transgenic mice, suggesting that enhancing AB degradation may be therapeutic in human AD. Insulin-degrading enzyme (IDE) is an Abeta-degrading zinc metalloprotease that requires a free thiol and bivalent cations to degrade extracellular Abeta in neurons and other cell types. The deduced sequence of IDE shares little homology to other proteinases, including cysteine, metallo-, serine, or aspartic proteases. Most IDE is localized inside the cell, where it can degrade internalized insulin, insulin-like growth factors I and II, and amylin, which make IDE an attractive target for type-2 diabetes. However, since IDE has also been found in the extracellular space and at the plasma membrane, it can function as a principal protease in Abeta catabolism. IDE secretion is not dependent upon the classical secretion pathway. Studies showing reduced IDE levels in human AD patients, combined with increased brain AB levels in IDE-deficient mice, and association studies suggesting that IDE variants may be associated with AD severity, suggest that the identification of compounds that selectively modulate IDE activity will present as important tools for the study of IDE function, AD, and diabetes.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Malcolm Leissring, Mayo Clinic College of Medicine
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R03 DA024888-01
Grant Proposal PI: Malcolm Leissring
External Assay ID: IDE_INH_FP_1536_1X%INH PRUN

iv. CHRM4

Description

To date, five muscarinic acetylcholine receptor (mAChR) subtypes have been identified (M1-M5) and play important roles in mediating the actions of ACh in the peripheral and central nervous systems. Of these, M1 and M4 are the most heavily expressed in the CNS and represent attractive therapeutic targets for cognition, Alzheimer's disease, and schizophrenia. In contrast, the adverse effects of cholinergic agents are thought to be primarily due to activation of peripheral M2 and M3 mAChRs. Due to the high sequence homology and conservation of the orthosteric ACh binding site among the mAChR subtypes, development of chemical agents that are selective for a single subtype has been largely unsuccessful, and in the absence of highly selective activators of M4, it has been impossible to test the role of selective M4 activation. Numerous pharmacological studies have provided evidence for a diversity of mAChR subtypes in brain and other tissues. These molecularly distinct receptors have important differences, including preferential coupling to various effector systems. In general, it is thought that coupling to Gq and phospholipase C is the predominant effector system coupled to M1, M3, and M5, whereas M2 and M4 most often couple to Gi/Go and inhibition of adenylyl cyclase or ion channels.

Clinical trials with xanomeline, a M1/M4-preferring orthosteric agonist, which also has sub-micromolar activity toward M5, demonstrated efficacy as both a cognition-enhancing agent and an antipsychotic agent. In follow-up studies in rats, xanomeline displayed an antipsychotic-like profile comparable to clozapine. However, a long standing question concerned whether or not the antipsychotic efficacy or antipsychotic-like activity in animal models is mediated by activation of M1, M4, or a combination of both receptors. In addition, xanomeline has been associated with unwanted gastrointestinal side effects and syncope that resulted in patient non-compliance during the trials. Data from mAChR knockout mice led to the suggestion that a selective M1 agonist would be beneficial for cognition, whereas an M4 agonist would provide antipsychotic activity for the treatment of schizophrenia. This proposal is further supported by recent studies demonstrating that M4 receptors modulate the dynamics of cholinergic and dopaminergic neurotransmission and that loss of M4 function results in a state of dopamine hyperfunction. These data, coupled with findings that schizophrenic patients have altered hippocampal M4 but not M1 receptor expression, suggest that selective activators of M4 may provide a novel treatment strategy for schizophrenia patients. However, multiple studies suggest that M1 may also play an important role in the antipsychotic effects of mAChR agonists and that the relative contributions of M1 and M4 to the antipsychotic efficacy of xanomeline or antipsychotic-like effects of this compound in animal models are not known. However, highly selective centrally penetrant activators of either M1 or M4 have not been available, making it impossible to determine the in vivo effects of selective activation of these receptors. Further, in vivo active tool compounds for the M5 receptor are also unavailable. These tools will prove invaluable for verifying that novel M1 or M4 compounds do not have off target effects on M5 in vivo and assist with determining the role of M5 in the CNS.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Colleen Niswender, Vanderbilt University School of Medicine
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: MH077606-01
Grant Proposal PI: Colleen Niswender, Vanderbilt University School of Medicine
External Assay ID: CHRM4_PAM_FLUO8_1536_1X%ACT PRUN

v. TTR

Description

The "Amyloid Hypothesis" of human Alzheimer#s disease (AD) is based on human genetic data, murine models in which mutant human Amyloid-B Precursor Protein (ABPP) and/or presenilins are expressed, and in vitro and tissue culture evidence indicating that aggregation of various forms of the neuronal Amyloid-BPP cleavage product Amyloid-B1-40/42 plays a major role in the pathogenesis of autosomal dominant and sporadic forms of human AD. It has been shown that genetic over-expression of wild type human TTR in the well-studied validated APP23 transgenic model of human AD suppresses the neuropathologic and behavioral AD phenotypes. Silencing the endogenous TTR gene accelerates the pathologic changes. A number of studies have shown that TTR and Amyloid-B interact, and that TTR inhibits Amyloid-B-induced cytotoxicity, Amyloid-B fibril formation and Amyloid-B oligomerization. Since neurons appear to use TTR as a protective molecule, enhancing neuronal production of TTR might be a useful strategy for either treatment or prophylaxis of AD. The overall goal of this project is to identify well characterized or novel compounds which can be used as pharmacologic agents to enhance neuronal transcription of the TTR gene (and subsequent TTR protein production) to determine if the same salutary pharmacological effect can be achieved is was possible genetically in a well-validated model of human Amyloid-B deposition.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Joel Buxbaum, The Scripps Research Institute (TSRI)
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: R21AG041496
Grant Proposal PI: Joel Buxbaum, The Scripps Research Institute (TSRI)

vi. Arfgap1

Description

The low molecular weight ADP-ribosylation factors (Arfs) regulate actin remodeling, vesicle trafficking, membrane lipid composition, and phospholipid metabolism. Arfs are members of the Ras family of GTP-binding proteins, switching between the GTP- and GDP-bound forms. Arf GTP binding and GTP hydrolysis is regulated by ARFGAPs (ARF GTPase-activating proteins) which associate with the Golgi apparatus and possess a conserved zinc-finger GAP catalytic domain. ARFGAPs such as ARFGAP1 and ASAP1 are found in cell structures involved in vesicle production and trafficking, adhesion, migration, and development. ARFGAP1 promotes hydrolysis of ARF1-bound GTP and is required for dissociation of coat proteins from Golgi-derived membranes and vesicles. ARFGAP1 is stimulated by phosphoinosides and inhibited by phosphatidylcholine. Dysfunctional regulation of ARFGAPs has been implicated in various diseases, including cancer, alzheimer disease, and autism. However, the catalytic mechanism and specific disease-associated roles of ARFGAPs are unclear, but recent studies suggest a role for Ca2+ in stimulating ARFGAP-mediated GTP hydrolysis. As a result the identification of modulators of ARFGAPs would provide useful tools to elucidate ARFGAP biology.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: University of North Carolina at Chapel Hill
Assay Provider: Qisheng Zhang, University of North Carolina at Chapel Hill
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1R21NS073041 (Fast Track)

Grant Proposal PI: Qisheng Zhang, University of North Carolina at Chapel Hill
External Assay ID: ARFGAP1_INH_FP_1536_1X%INH PRUN

vii. MAPT

Description

Assay Provider: Carlo Ballatore, University of Pennsylvania
The microtubule-associated protein tau is an abundant protein in the axons of neurons that stabilizes microtubules. With its ability to modulate microtubule dynamics, tau contributes directly or indirectly, to key structural and regulatory cellular functions. Particularly important is the influence tau exerts on axonal transport, which allows signaling molecules, trophic factors and other essential cellular constituents to travel along the axons. Under pathological conditions, tau becomes sequestered into insoluble aggregates called neurofibrillary tangles. This phenomenon is believed to have pathological consequences by promoting axonal transport deficits that ultimately lead to synaptic dysfunction and neuronal loss. To identify inhibitors of tau aggregation, a heparin-induced tau fibril formation assay was used that employed a recombinantly expressed fragment of tau, K18 (Q242-E372), bearing a P301L mutation (Gustke et al. 1994; Hong et al. 1998). This assay monitors tau fibrillization by Thioflavin T (ThT) fluorescence.

NIH Chemical Genomics Center [NCGC]
MLPCN Grant: X01 MH083262-01
Assay Provider: Carlo Ballatore, University of Pennsylvania

viii. SLC5A7 choline transporter

Description

In the brain, the chemical acetylcholine (ACh) exerts powerful modulatory control over arousal, motor and cognitive circuits, and has been found to be deficient in Alzheimer's Disease (AD). The current drugs available to positively impact cognitive deficits in Alzheimer's Disease (AD) and other dementias are the cholinesterase inhibitors. These prevent the breakdown of the neurotransmitter acetylcholine (ACh), and thus augment Ach function. Due to the limited utility of the cholinesterase inhibitors, alternative therapies to augment ACh deficits are critical in our aging population.
Another vital protein, the hemicholinium-3 sensitive choline transporter (CHT) is believed to be responsible for the efficient uptake of choline by neurons to allow for ACh synthesis. An assay system for high throughput screening has been developed to identify compounds with high selectivity for CHT. It is anticipated that these compounds may lead to future cholinergic therapies in AD, and multiple other CNS diseases regulated by cholinergic signaling. These compounds may be able to modulate choline uptake and the levels of ACh produced in the neuron by impacting the kinetics of neurotransmitter synthesis. Such reagents would provide useful probes for the role of this transporter in normal and diseased states.
Principle of the assay
This HTS assay is a choline-induced membrane potential assay measuring choline coupled sodium flow through CHT. The kinetic relationship between sodium and choline in the transporter is not well established, but dephosphorylation of the transporter is associated with a decrease in the sodium current and a decrease in the km of transport. Allosteric modulation of CHT may mimic this finding and induce a higher efficiency kinetic state of CHT. Compounds that decrease the signal of the choline-induced membrane potential assay at this choline concentration will be selected to retest for choline uptake.
The objective of the current screen is to identify compounds that inhibit the choline induced membrane depolarization of cells by CHT using a HEK293 cell line which stably expresses

choline transporter (CHT). Compounds selected as CHT inhibitors will later be counter-screened for specificity.

Data Source (MLPCN Center Name): Johns Hopkins Ion Channel Center (JHICC)
Center Affiliation: Johns Hopkins University, School of Medicine
Screening Center PI: Min Li, Ph.D.
Assay Provider: Alicia Ruggiero, Ph.D., Vanderbilt University Medical Center
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1R03DA028852-01
Grant Proposal PI: Alicia Ruggiero, Ph.D., Vanderbilt University Medical Center
Assay Implementation: Zhihong Lin Ph. D., Xiaofang Huang M.S., Shunyou Long M.S., David Meyers Ph.D., Owen McManus Ph.D., and Meng Wu Ph.D.
HTS execution: Xiaofang Huang M.S., Zhihong Lin Ph. D., Shunyou Long M.S., and Meng Wu Ph.D.

b. ALS

i. TDP-43

Description

The goal of this project is to identify new chemical probes capable of reversing the toxicity associated with TDP-43 which is associated with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. These compounds will provide a complementary approach to deciphering the tangled mechanisms of toxicity and protection that we have uncovered through genome-wide genetic screens and are beginning to uncover in a large-scale proteomics analysis with mass spectroscopy.

To this end, a miniaturized assay was developed to be screened against the Molecular Libraries Small Molecule Repository (MLSMR) to discover TDP-43 inhibitors. Please note that in the set-up this yeast-based assay compounds that inhibit as TDP-43, have a read-out as activators.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: NS060957
Assay Submitter (PI): Susan Lindquist, Whitehead Institute/MIT

c. ALS, AD, neurodegeneration

i. mPTP

Description

Given its multifactorial roles, regulation of cellular Ca2+ metabolism and bioenergetics functions as an integrated system. In terms of normal physiology, this integration is reflected in mitochondrion's high capacity to store Ca2+ which may protect cells like neurons against transient elevation in intracellular Ca2+ during periods of hyperactivity. Furthermore, mitochondrial release of Ca2+ can amplify and sustain signals arising from elevation of cytoplasmic Ca2+ in response to extracellular events. An additional consequence of mitochondrial Ca2+ accumulation is the stimulation of oxidative metabolism through the activation of matrix Ca2+-sensitive dehydrogenases. As a result, mitochondrial Ca2+ homeostasis is tightly regulated and is based in a series of specific uptake and release

systems. Importantly, regulation of ion fluxes across mitochondrial membranes, specifically the inner mitochondrial membrane (IMM), is essential since energy is stored in the form of a proton electrochemical potential difference which is used to drive both ATP synthesis and mitochondrial Ca2+ uptake and release.

One mitochondrial Ca2+ efflux pathway is represented by the mitochondrial permeability transition pore (mtPTP) which, in vitro, results in an IMM permeability increase to solutes with molecular masses of about 1,500 Da or lower. A great deal of information is available about the functional properties of the mtPTP.

In intact cells under normal conditions, mtPTP opens only transiently (and reversibly). These transient states likely mediate the fast release of Ca2+ from mitochondria in response to normal physiological signals that raise cytosolic, and hence mitochondrial Ca2+ levels to those required for mtPTP activation (the "threshold"). However, under pathological conditions, persistent activation of the mtPTP has dramatic consequences on cellular and mitochondrial function. This mode of activation results in the collapse of the membrane potential across the IMM (required to drive mitochondrial accumulation of Ca2+ and the synthesis of ATP) and depletion of pyridine nucleotides and respiratory substrates, causing respiratory inhibition and cell death. Consequently, mtPTP has long been implicated as a target for mitochondrial dysfunction in vivo, particularly in the context of specific human pathological events.

The goal of this high-throughput assay is to identify compounds that inhibit mtPTP. This is accomplished via the measurement of the change in absorbance of freshly isolated mitochondria in assay buffer due to swelling which occurs via the uptake of Ca2+ in the presence of test compounds.

Data Source: Sanford-Burnham Center for Chemical Genomics (SBCCG)
Source Affiliation: Sanford-Burnham Medical Research Institute (SBMRI, San Diego, CA)
Network: NIH Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Number: 1 R03 MH096534-01
Assay Provider: Michael Forte, Ph.D., Oregon Health & Science University, Portland, OR

ii. P97 ATPase

Description

Misfolded proteins accumulate in the endoplasmic reticulum (ER) in response to environmental stress. To reduce the burden these proteins place on the secretory pathway, eukaryotic cells have evolved a process, known as ER-associated degradation (ERAD), to recognize and eliminate these proteins. The highly conserved p97 ATPase functions in ERAD by hydrolyzing ATP needed to export ubiquitinated substrates to the cytosol for degradation by the proteasome. The discovery of p97 missense mutations in a genetic form of human dementia, the localization of p97 in ubiquitylated inclusions in affected neurons of amyotrophic lateral sclerosis (ALS) and Parkinson's disease, and the overproduction of p97 in multiple cancers, suggests that p97 has diverse and essential cellular roles. Thus, the identification of probes that selectively target p97 activity may provide insights into the biological roles of p97.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: Raymond Deshaies, California Institute of Technology
Network: Molecular Libraries Probe Production Centers Network (MLPCN)

Grant Proposal Number 1 R03 MH085687-01
Grant Proposal PI: Raymond Deshaies, California Institute of Technology
External Assay ID: P97_INH_Lumi_1536_%INH

d. HD, AD, PD, ALS, neurodegeneration

i. Heat shock 70kDa protein 1A

Description

The human heat shock protein 70 (Hsp70) family is evolutionarily conserved among all organisms from archaebacteria to humans, suggesting an essential role in cell survival (1, 2). Under circumstances of transient cell stress, the heat shock response and activities of molecular chaperones can restore protein homeostasis. In human disease, however, misfolded proteins can accumulate when polyglutamine-expansion proteins are chronically expressed over the life of the cell. Elevated expression of molecular chaperones suppresses protein misfolding/aggregation and toxicity phenotypes in various model systems of Huntington's disease, Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS). Mutations in the respective proteins huntingtin, tau, alpha-synuclein, and superoxide dismutase (SOD1), associated with these diseases, result in the appearance of misfolded species that adopt alternate conformations. These observations led to the proposal that a common feature of diverse diseases of protein conformation is the appearance of alternate folded states that self-associate and form toxic species and protein aggregates.

A role for Hsp70 family proteins in controlling these events has been widely studied. Studies with mammalian tissue culture cells, transgenic mice, Drosophila, and C. elegans have established that the heat shock response can be activated in cells expressing aggregation-prone proteins, suggesting a role for molecular chaperones as an adaptive survival response (3, 4). Moreover, a direct relationship with polyglutamine diseases is suggested by the co-localization of several heat shock proteins, including Hdj-1, Hdj-2, Hsp70 and ubiquitin with polyglutamine aggregates in the tissues of affected individuals, transgenic mice and tissue culture cells (5). Finally, overexpression of Hsp70 can suppress the toxicity associated with the accumulation of misfolded proteins (6-8). High throughput screening initiatives aimed at the identification of compounds that enhance the heat shock response, in particular Hsp70, will provide insights into this conserved cellular process and may lead to novel therapeutics for these devastating disorders.

Source (MLSCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)
Assay Provider: Richard Morimoto, Northwestern University
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number: 5 R21 NS056337-02
Grant Proposal PI: Richard Morimoto

e. PD

i. TAAR1

Description

Heterotrimeric G-protein coupled receptors (GPCRs) are major targets for disease therapeutics, due in part to their broad tissue distribution, structural diversity, varied modes of action, and disease-associated mutations. TAAR1 (trace amine associated receptor 1) is a G protein-coupled receptor activated by trace amines. Trace amines (TA) such as Beta-phenethylamine (Beta-PEA), p-tyramine (TYR), octopamine, and tryptamine are endogenous amine compounds that account for less than 1% of the biogenic amines in most brain regions, and exert pharmacological actions in humans. In addition to binding Beta-PEA and TYR, rat TAAR1 is also activated by dopamine, octopamine, tryptamine, amphetamine, and lysergic acid. TAs are of particular interest because they have been shown to modulate the activity of neurotransmitters such as dopamine and gamma-amino butyric acid and alterations in their brain levels are associated with schizophrenia and depression. Their potential to modulate dopaminergic activity suggests that they may play a role in the efficacy of L-DOPA in treating Parkinson disease4 and addiction. In humans, only TAAR1 has been shown to be activated by Beta-PEA and TYR, resulting in increased cyclic adenosine monophosphate (cAMP) accumulation through coupling to Gs. Endogenous hTAAR1 activity is predominantly coupled to G-alpha-s and the accumulation of cAMP. The variety of compounds potentially acting at this receptor makes it attractive to assume that hTAAR1 is involved in a variety of integrated CNS processes such as mood and cognition. As a result, hTAAR1 is an interesting target for the development of ligands to probe the role of this receptor in CNS function and disease. For this project, the assay provider has created a cell line expressing the hTAAR1 in a parent cell line (RD-HGA16 cells, Molecular Devices) that stably over expresses the promiscuous G-protein, G-alpha-16, thereby coupling hTAAR1 activation to mobilization of internal calcium stores. Because TAs may be involved in modulating a variety of behaviors including mood, cognition, and addiction, it is of interest to discover novel ligands for TAAR1 to probe the role TAs play in brain function.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: RTI International
Assay Provider: Brian P. Gilmour, RTI International
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1R21NS064780-01A1
Grant Proposal PI: Brian P. Gilmour, RTI Internationall
External Assay ID: TAAR1_ANT_FLUO8_1536_1X%INH PRUN

ii. D1

Description

Dopamine receptors have been classified into two large families, the D1-like and the D2-like (Neve et al., 2004). Members of the D1-like receptor family include D1 and D5 dopamine receptors. Activation of D1-like receptors stimulate Gs which in turn activates adenylate cyclase resulting in enhanced cyclic AMP accumulation (Neve et al., 2004). Members of the D2-like receptor family include D2, D3, and D4 dopamine receptors. Activation of D2-like dopamine receptors is often linked to inhibition of drug-stimulated cyclic AMP accumulation (Watts et al., 1999, Watts et al., 1998). The field of dopamine research greatly benefited in the latter part of the last century by the availability of dopamine D2 agonists and antagonists. Indeed, the 'dopamine hypothesis' of schizophrenia resulted directly from the availability of D2 dopamine antagonists and their value in attenuating the symptoms of schizophrenia. Intense research into the importance of D2-like receptors resulted in a large variety of D2-like agonists and antagonists. By contrast, dopamine D1 (D1A)receptors had not been considered important until about 15 years ago, and no one today yet knows the importance of D5 (D1b) dopamine receptors.

The discovery of D1 dopamine receptor-selective allosteric modulators would certainly provide important tools for neuropharmacology (Schetz, 2005). Although an individual allosteric modulator could either reduce or enhance the agonist-stimulated receptor signal, an allosteric potentiator of D1 receptor signaling is highly desirable. There are several potential clinical uses for allosteric potentiators of D1 receptor signaling that relate to the treatment of Parkinson#s disease (PD). For example, an allosteric potentiator of D1 receptors could serve as the drug of first choice in the initial stages of PD. This allosteric potentiator would serve to enhance the functional activity of the remaining levels of dopamine in the striatum. Moreover, an allosteric potentiator would be activity-dependent and have actions only during the presence of released dopamine providing a more natural pattern of D1 dopamine receptor activation. An allosteric potentiator of D1 receptors could also be used in combination therapy with L-DOPA. This therapeutic approach would allow clinicians to initiate dopamine-replacement therapy with lower doses of L-DOPA, reducing immediately the important and dose-limiting side effects on the cardiovascular system (i.e. arrhythmias and hypotension) as well as the gastrointestinal system (i.e. nausea and vomiting).

Assay Provider: Val Watts
Assay Provider Affiliation: Purdue University
Grant Title: Allosteric Modulators of D1 Receptors
Grant Number: 1 X01 MH077619-01

7. Mood disorders

a. Anxiety

i. TRH receptor enhancers

Description

This project aims to identify small drug-like molecules that activate (agonists) the thyrotropin releasing hormone (TRH) receptor (TRHR) or enhance its TRH-stimulated activity (allosteric enhancers). TRHR, a drug-accessible cell surface receptor expressed in specific brain regions, and its ligand TRH have been implicated in several central nervous system disorders, including depression and anxiety. Small molecule TRHR agonists or allosteric enhancers that are metabolically stable and able to cross the blood-brain barrier might serve as probes to elucidate the roles of brain TRH receptors in animal models, and might be valuable as lead compounds for the development of drugs to treat patients with central nervous system disorders.

Upon TRHR-Gq activation in TRHR cells, intracellular calcium is released from stores in the endoplasmic reticulum. A cytosolic calcium indicator exhibits increased fluorescence in the presence of calcium release. This primary screen measures compound-induced enhancement of an EC20 stimulation of the receptor by its native ligand.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH089816-01
Assay Submitter (PI): Marvin Gershengorn

ii. NPS receptor antagonists

Description

Neuropeptide S receptor (NPSR), previously known as GPR154, is a recently de-orphanized G protein coupled receptor. Its endogenous ligand is the 20 amino acids peptide Neuropeptide S (NPS). Activation of NPSR induces transient increases in intracellular calcium and cAMP, suggesting coupling of this receptor to both Gs and Gq G proteins. NPS and its receptor are found in various tissues. Specifically they are highly expressed in brain areas that have been implicated in modulation of arousal, stress and anxiety. Central administration of NPS in mice produces an unusual profile of activity by inducing wakefulness and arousal, while at the same time suppressing anxiety. Therefore, NPSR may represent a novel drug target for the treatment of sleep and anxiety disorders.

To identify NPSR antagonists, we developed a cell-based cAMP assay. NPS can stimulate the production of cAMP in Chinese hamster ovary cells stably expressing NPS receptor. This change in intracellular cAMP level can be detected by a homogeneous LANCE cAMP assay based on the TR-FRET (time resolved fluorescence resonance energy transfer) between a europium-labeled cAMP tracer complex and a cAMP-specific antibody labeled with Alexa Fluor 647. The europium-labeled cAMP tracer complex is formed by the tight interaction between Biotin-cAMP (b-cAMP) and streptavidin labeled with Europium-W8044 chelate (Eu-SA). Light pulse at 320 nm excites the europium of the cAMP tracer and the energy emitted is transferred to the Alexa molecule bound to the cAMP antibody, generating a TR-FRET signal at 665 nm. Residual energy from the europium will produce a light at 620 nm. The native unlabeled cAMP from cell lysates competes with the europium-cAMP tracer for antibody binding and reversely reduces the emission signal of Alexa by interrupting FRET between the two labeled molecules. Both emission signals from the FRET donor (620 nm) and the acceptor (665 nm) can be detected by a plate reader in the TRF mode. Expression of result in fluorescence ratio (665 nm/620 nm) helps to normalize differences due to cell density and reagent dispensing. This assay was successfully optimized to a 1536-well plate format.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production centers Network [MLPCN]
MLPCN Grant: X01-DA026210-01
Assay Submitter (PI): Heilig, Markus Alexander

iii. NPY1 receptor antagonists

Description

Neuropeptide Y (NPY) is a neurotransmitter with physiologic roles including control of feeding behavior, regulation of cortical neural activity, heart neural activity, and emotional regulation. Importantly, NPY is implicated in human diseases such as obesity, depression and alcoholism. NPY mediates its biological effects in part through activation of the Galphai protein coupled receptors (GPCRs) NPY-Y1 and Y2 receptors, which decrease cytosolic cAMP production. Recent studies have implicated these receptors in diverse biological events, including feeding, alcoholism, anxiety and depression, pain perception, immunity and inflammation, vascular remodeling, hypothermia, pancreatic islet cell function, bone and energy metabolism, and tumorigenesis. Due to the varied role of these receptors in human disease and physiology, the identification of high affinity selective probes that target each receptor subtype may provide novel tools for the study of NPY-related pathologies.

Source (MLSCN Center Name): The Scripps Research Institute Molecular Screening Center
Center Affiliation: The Scripps Research Institute (TSRI)

Assay Provider: Claes Wahlestedt, TSRI
Network: Molecular Library Screening Center Network (MLSCN)
Grant Proposal Number 1 R21 NS056950-01
Grant Proposal PI: Claes Wahlestedt

iv. galanin receptor antagonists

Description

Galanin, a 29 amino acid neuropeptide (30 residues in humans), is cleaved from preprogalanin and is involved in many physiological processes including nervous system development, feeding, metabolism and reproduction, and regulation of neurotransmitter and hormone release (1, 2). The physiologic response to galanin is mediated in part by three G protein-coupled metabotropic 7-transmembrane receptor subtypes, GalR1, GalR2 and GalR3. These receptors are expressed throughout the peripheral and central nervous systems as well as the endocrine system. Both GalR1 and GalR2 are widely expressed in the CNS whereas GalR3 is the least abundantly expressed of the galanin receptor subtypes. The GalR3 in particular has been strongly implicated in addiction and mood related disorders such as anxiety and depression. It has been the target of many drug discovery programs within the pharmaceutical industry but despite the significant resources and effort devoted to discovery of galanin receptor subtype selective small molecule modulators, there have been very few reports for the discovery of such molecules and in addition, all access to primary screening data remains proprietary information. As a result the identification of novel GalR3 antagonists would serve as useful tools for understanding galanin biology and for possible investigations into the role of GalR3 in addiction and mood disorders (3-5).

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Patricia McDonald, The Scripps Research Institute
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R21 NS067631-01
Grant Proposal PI: Patricia McDonald, The Scripps Research Institute
External Assay ID: GALR3_ANT_CNGC_1536_1X%INH PRUN

b. Drug addiction

i. dopamine transporter

Description

Cocaine abuse and addiction continue to be a problem around the world. Currently, there are no effective pharmacological treatments for cocaine addiction. Cocaine binds and inhibits the transporters for neurotransmitters dopamine, serotonin and norepinephrine. Ample evidence suggests that cocaine inhibition of dopamine transporter (DAT) primarily mediates cocaine's rewarding and addictive effects. Cocaine does not produce reward in knock-in mice with a cocaine-insensitive DAT mutant, indicating that cocaine inhibition of DAT is necessary for cocaine reward. Therefore, compounds that antagonize cocaine inhibition of DAT have great potentials as lead compounds for the development of effective drugs treating cocaine addiction. There have been significant efforts in the past to search for cocaine antagonists. Several classes of compounds have been found that antagonize cocaine binding to DAT, but these compounds are DAT inhibitors themselves and likely to be potentially addictive too. Successful identification of a compounds that antagonize cocaine's

effect on DAT may lead to the development of the first specific pharmacological treatment for cocaine addiction.

Screening Center: Vanderbilt Screening Center for GPCRs, Ion Channels and Transporters
Assay Provider: Howard Gu
Assay Provider Affliation: Ohio State University
Grant Title: High Throughput Screening for Cocaine Antagonists
Grant Number: 1X01MH079819-01

c. Schizophrenia

i. TAAR1

Description

Heterotrimeric G-protein coupled receptors (GPCRs) are major targets for disease therapeutics, due in part to their broad tissue distribution, structural diversity, varied modes of action, and disease-associated mutations. TAAR1 (trace amine associated receptor 1) is a G protein-coupled receptor activated by trace amines. Trace amines (TA) such as Beta-phenethylamine (Beta-PEA), p-tyramine (TYR), octopamine, and tryptamine are endogenous amine compounds that account for less than 1% of the biogenic amines in most brain regions, and exert pharmacological actions in humans. In addition to binding Beta-PEA and TYR, rat TAAR1 is also activated by dopamine, octopamine, tryptamine, amphetamine, and lysergic acid. TAs are of particular interest because they have been shown to modulate the activity of neurotransmitters such as dopamine and gamma-amino butyric acid (14-16) and alterations in their brain levels are associated with schizophrenia and depression. Their potential to modulate dopaminergic activity suggests that they may play a role in the efficacy of L-DOPA in treating Parkinson disease4 and addiction. In humans, only TAAR1 has been shown to be activated by Beta-PEA and TYR, resulting in increased cyclic adenosine monophosphate (cAMP) accumulation through coupling to Gs. Endogenous hTAAR1 activity is predominantly coupled to G-alpha-s and the accumulation of cAMP. The variety of compounds potentially acting at this receptor makes it attractive to assume that hTAAR1 is involved in a variety of integrated CNS processes such as mood and cognition. As a result, hTAAR1 is an interesting target for the development of ligands to probe the role of this receptor in CNS function and disease. For this project, the assay provider has created a cell line expressing the hTAAR1 in a parent cell line (RD-HGA16 cells, Molecular Devices) that stably over expresses the promiscuous G-protein, G-alpha-16, thereby coupling hTAAR1 activation to mobilization of internal calcium stores. Because TAs may be involved in modulating a variety of behaviors including mood, cognition, and addiction, it is of interest to discover novel ligands for TAAR1 to probe the role TAs play in brain function.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: RTI International
Assay Provider: Brian P. Gilmour, RTI International
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1R21NS064780-01A1
Grant Proposal PI: Brian P. Gilmour, RTI Internationall
External Assay ID: TAAR1_ANT_FLUO8_1536_1X%INH PRUN

ii. D(3) dopamine receptor

Description

D3 DARs represent a very important target for the treatment of several neuropsychiatric disorders. Indeed, one of the most promising therapeutic applications for the D3 DAR is in the area of addiction and related disorders. Several lines of evidence suggest that partial agonists or antagonists of the D3 DAR may be therapeutic for drug abuse and relapse. Accumulating evidence suggests that reducing D3 DAR activity may regulate the motivation to self-administer drugs and disrupt drug-associated cue-induced craving and relapse or reinstatement of drug taking. These findings have been observed with a number of addictive substances including cocaine, amphetamine, nicotine, and alcohol. It is particularly interesting that partial agonists are effective in these preclinical models, suggesting that only partial blockade of the D3 DAR is needed and may, in fact, prove to be more beneficial than full blockade. Antagonism of the D3 DAR may also be therapeutic in the treatment of schizophrenia or psychosis. Notably, while all antipsychotic drugs block the D2 DAR, they also block the D3 DAR to various degrees. Since the D3 DAR is expressed in areas of the CNS associated with the control of mood and emotion, it has been hypothesized that selective antagonism of the D3 DAR may be effective in treating psychosis without inducing the motor side effects typically seen with D2 DAR antagonists. Interestingly, D3 DAR antagonism has also been suggested to be highly beneficial in the treatment of certain motor/movement disorders such as L-DOPA-induced dyskinesias, which typically arise during late-stage Parkinson's disease treatment.

The goal of this project is to use high throughput screening approaches to identify and develop novel, highly selective small molecule allosteric modulators of the D3 DAR for use as in vitro and in vivo pharmacological tools and in proof-of-concept experiments in animal models of neuropsychiatric disease. This part of the project aims to discover novel antagonist.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH094203
Assay Submitter (PI): David Sibley, NINDS

iii. Wnt/Beta-catenin pathway

Description

The canonical Wnt signaling pathway regulation is thought to be critical in in several disorders, including bipolar disorder and schizophrenia as well as in embryonic brain development and adult neurogenesis. Signaling through the Wnt pathway leads to the accumulation and nuclear translocation of a-catenin, and the transcriptional activation of genes with promoters containing TCF/LEF response elements that bind the TCF/LEF family of transcription factors. To further interrogate the Wnt signaling pathway, a neuronal progenitor cell (NPC) line stabally expressing a TCF/LEF luciferase reporter system (NPC-TCF/LEF) stabally expressed was developed. NPC-TCF/LEF cells were grown in a 384-well plate format in the presence of an EC25 Wnt3a-conditioned media (Wnt3a-CM).AnEC25 of Wnt3a-CM was chosen to be able to sensitively identify activators of the endogenous Wnt/a-catenin signaling without reaching a ceiling effect that may obscure the activity of strong activators. CHIR-99021, which gives ~5-fold activation under conditions of EC25 of Wnt3a-CM, was used as a positive control. Increase in TCF/LEF luciferase reporter activity was detected using Steady-Glo luciferase assay.

Expected Outcome: Compounds that increase luminescence will be considered 'actives' increasing TCF/LEF reporter system

Source: Broad Institute
Grant Number: R21/R33MH087896-03
Primary Collaborators: Steve Haggarty,Stanley Center,haggarty@broadinstitute.org
Wendy Zhao,Stanley Center,wzhao@chgr.mgh.harvard.edu

iv. KCNH2

Description

The recently identified KCNH2 3.1 potassium channel, a brain selective isoform of the KCNH2 (hERG1) potassium channel, has been shown to be increased in the brains of patients with schizophrenia, a genetic risk factor for the emergence of schizophrenia, and to affect neuronal cell activity and brain physiology. Many antipsychotic drugs that inhibit the neurotransmitter dopamine also bind to hERG1 channel which might help explain their antipsychotic activity but also produces cardiac side effects. The discovery of the novel KCNH2 3.1 isoform offers a potential new target for the development of antipsychotic drugs without cardiac side effects. This proposal will use a high throughput thallium flux assay developed for KCNH2 to screen compounds that modulate the activity of KCNH2 3.1. The wild type KCNH2 channel will also be screened in parallel to help define the selective modulators of this KCNH2 3.1 potassium channel. The resulting compounds will be validated in electrophysiology experiments. The availability of animal models will allow future testing in vivo for effects on memory and other aspects of animal physiology linked with psychosis.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: MH096539
Assay Submitter (PI): James Barrow, Johns Hopkins University, Lieber Institute for Brain Development

v. CHRM4

Description

To date, five muscarinic acetylcholine receptor (mAChR) subtypes have been identified (M1-M5) and play important roles in mediating the actions of ACh in the peripheral and central nervous systems. Of these, M1 and M4 are the most heavily expressed in the CNS and represent attractive therapeutic targets for cognition, Alzheimer's disease, and schizophrenia. In contrast, the adverse effects of cholinergic agents are thought to be primarily due to activation of peripheral M2 and M3 mAChRs. Due to the high sequence homology and conservation of the orthosteric ACh binding site among the mAChR subtypes, development of chemical agents that are selective for a single subtype has been largely unsuccessful, and in the absence of highly selective activators of M4, it has been impossible to test the role of selective M4 activation. Numerous pharmacological studies have provided evidence for a diversity of mAChR subtypes in brain and other tissues. These molecularly distinct receptors have important differences, including preferential coupling to various effector systems. In general, it is thought that coupling to Gq and phospholipase C is the predominant effector system coupled to M1, M3, and M5, whereas M2 and M4 most often couple to Gi/Go and inhibition of adenylyl cyclase or ion channels.

Clinical trials with xanomeline, a M1/M4-preferring orthosteric agonist, which also has sub-micromolar activity toward M5, demonstrated efficacy as both a cognition-enhancing agent and an antipsychotic agent. In follow-up studies in rats, xanomeline displayed an antipsychotic-like profile comparable to clozapine. However, a long standing question concerned whether or not the antipsychotic efficacy or antipsychotic-like activity in animal models is mediated by activation of M1, M4, or a combination of both receptors. In addition, xanomeline has been associated with unwanted gastrointestinal side effects and

syncope that resulted in patient non-compliance during the trials (10). Data from mAChR knockout mice led to the suggestion that a selective M1 agonist would be beneficial for cognition, whereas an M4 agonist would provide antipsychotic activity for the treatment of schizophrenia. This proposal is further supported by recent studies demonstrating that M4 receptors modulate the dynamics of cholinergic and dopaminergic neurotransmission and that loss of M4 function results in a state of dopamine hyperfunction. These data, coupled with findings that schizophrenic patients have altered hippocampal M4 but not M1 receptor expression, suggest that selective activators of M4 may provide a novel treatment strategy for schizophrenia patients. However, multiple studies suggest that M1 may also play an important role in the antipsychotic effects of mAChR agonists and that the relative contributions of M1 and M4 to the antipsychotic efficacy of xanomeline or antipsychotic-like effects of this compound in animal models are not known. However, highly selective centrally penetrant activators of either M1 or M4 have not been available, making it impossible to determine the in vivo effects of selective activation of these receptors. Further, in vivo active tool compounds for the M5 receptor are also unavailable. These tools will prove invaluable for verifying that novel M1 or M4 compounds do not have off target effects on M5 in vivo and assist with determining the role of M5 in the CNS.

Source (MLPCN Center Name): The Scripps Research Institute Molecular Screening Center (SRIMSC)
Affiliation: The Scripps Research Institute, TSRI
Assay Provider: Colleen Niswender, Vanderbilt University School of Medicine
Network: Molecular Library Probe Production Centers Network (MLPCN)
Grant Proposal Number: MH077606-01
Grant Proposal PI: Colleen Niswender, Vanderbilt University School of Medicine
External Assay ID: CHRM4_PAM_FLUO8_1536_1X%ACT PRUN

8. Rare Diseases

a. cystic fibrosis

i. CFTR

Description

Cystic fibrosis (CF) is an autosomal recessive disease, caused by mutations in the gene coding for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). About 90% of people with CF have at least one copy of the "F508del" mutation (deletion of phenylalanine at position 508, or CFTR-F508del), and over 70% of CF patients are homozygous for this mutation. CFTR-F508del proteins are improperly folded, defective in trafficking to the plasma membrane, which results in the impairment of the channel functions. The mutated channel is still functional but is not present at the cell surface to achieve its normal functions. This assay aims to evaluate the correcting activity of small molecules on the function of CFTR-F508del. The correcting activity is determined by examining the fluorescence quenching ability of human bronchial epithelial CFBE cells expressing CFTR-F508del as well as a halide-sensitive Yellow Fluorescent Protein (YFP). Compound(s) restoring CFTR-F508del function will allow additional mutated CFTR channels to be expressed at the cell surface resulting in an enhanced fluorescent quenching ability of these CFBE cells.

Expected outcome: Identify small molecules decreasing the fluorescence mediated by a halide-sensitive YFP present in CFBE cells expressing a mutated form of the human CFTR protein (F508del) by the quencher sodium iodide. Compounds reducing the fluorescence further down compared to the neutral control by at least 8% in duplicate will be considered as active hits.

Source: Broad Institute
Grant Number: 1 R03 MH097529-01
Primary Collaborators: Jianling Sui,Flatley Discovery Laboratory,Charlestown, MA, jinlsui@flatleydiscoverylab.com.

ii. Ano1

Description

Recent discovery of TMEM16A which encodes a calcium-activated chloride channel (CaCC) has provided a new pathway to identify novel small molecule probes for this important and elusive target. Abnormality of CaCC activity is thought to be causal or linked to several major diseases, including cystic fibrosis, asthma, chronic bronchitis, and hypertension. Its roles are implicated in numerous physiological processes including cardiac and neuronal excitation, sensory transduction, trans-epithelial secretion, smooth muscle contraction, and fertilization. Discovery of CaCC-specific chemical probes can provide not only useful tool molecules for pharmacological interrogation of the CaCC function and diversity, but may also provide compounds as starting points for therapeutic efforts to treat various diseases resulting from dysfunction in chloride transport.

Our objective is to find potent and specific small molecule chemical probes that modulate TMEM16A-based calcium-activated chloride channel, by performing a primary screen of the MLSMR library of 300,000 compounds to identify small molecules and structural scaffolds that modulate this calcium-activated chloride channel; and examining and identifying hits with preferential effects on TMEM16A versus other chloride channels after validation, confirmatory and counter-screens. The intended applications of the small molecule probe will be for: (a) the in vitro electrophysiological studies of CaCC functions and modulation, without the limitations of non-specific interactions with related channels; (b) dissection and delineation of the various CaCC currents in multiple tissues; (c) evaluation of potential in vivo applications for rescue of defective chloride transport in cystic fibrosis.

Despite the discovery of the molecular identity of the CaCC channel, it is broadly recognized that there are far less CaCC/TMEM16A specific activators/potentiators available than inhibitors. Most of reported CaCC activators have been defined are non-specific potentiators, which increase the intracellular Ca2+ and consequentially indirectly affect CaCC. Only until very recently that CaCC (TMEM16A) specific activators without Ca2+ modulation have yet to be reported.

Principle of the assay
The iodide ion, which is permeable through the TMEM16A channel, serves as a surrogate for Cl-flux. The iodide flux is detected by YFP Iodide Biosensors (YFP mutant at H148Q/ I152L). The activation of the TMEM16A by ionomycin (or ATP) triggers the channel opening and I- influx, and consequentially the quenching of the mutant YFP fluorescence. Any compounds which activate/potentiate the TMEM16A channels will result in increased quenching and lower remaining fluorescence as detected by imaging plate reader FDSS.

Assay overview:

The purpose of this assay is to identify test compounds that activate/potentiate the calcium-activated chloride channel (TMEM16A). This assay employs a HEK293 cell line that stably expresses TMEM16A channels. The cells are treated with test compounds, followed by measurement of intracellular iodide, as monitored by a iodide-sensitive fluorescent protein YFP mutant at H148Q/ I152L. Those HEK293 cells stably expressing TMEM16A channels were plated into 384-well plates. On the following day, cells were incubated with assay buffer after removing media. Compounds were added to the assay buffer. Cells were incubated with 10 uM compound for 20 minutes, and fluorescence changes measured upon the addition of trigger solution (16 mM iodide final in the well). The fluorescence of YFP was measured on a Hamamatsu FDSS 6000 kinetic imaging plate reader. Compound effect was evaluated by the calculated YFP fluorescence ratio, normalized with negative controls. If the compound causes less than negative 3 times the standard deviation of the B-scores of the library compounds, the compound is then considered to be active as an activator/potentiator of the TMEM16A calcium-activated chloride channels.

Data Source: Johns Hopkins Ion Channel Center (JHICC_CaCC_Act_Primary)
BioAssay Type: Primary, Primary Screening, Single Concentration Activity Observed, Duplicate
Source (MLPCN Center Name): Johns Hopkins Ion Channel Center (JHICC)
Center Affiliation: Johns Hopkins University, School of Medicine
Screening Center PI: Min Li, Ph.D.
Assay Provider: Meng Wu, Ph.D.
Network: Molecular Libraries Probe Production Centers Network (MLPCN)
Grant Proposal Number: 1 R03 DA031670-01
Grant Proposal PI: Meng Wu, Ph.D., Johns Hopkins University School of Medicine
Assay Implementation: Zhihong Lin Ph.D., Hongkang Zhang, Kaiping Xu M.S., Shunyou Long M.S., Jing Zhang, Ph.D., Yixin Zhang, Alison Neal, Owen McManus Ph.D., and Meng Wu Ph.D.
HTS execution: Zhihong Lin Ph.D., Kaiping Xu M.S., Shunyou Long M.S., Jing Zhang, Ph.D., Yixin Zhang, and Meng Wu Ph.D.

b. McCune-Albright syndrome (MAS)

i. GNAS

Description

McCune-Albright syndrome (MAS) is a rare disease caused by somatic mutations in Gsgsp), a widely expressed protein central in signal transduction from G-coupled protein receptors (GPCRs). The disease symptoms are diverse, owing to the fact that the patients are mosaics with varying degrees of tissue involvement. Greater than 90% of the mutations in Gs in MAS occur at the R201 position and are equally divided between R201H and R201C. The R201 residue is central in the intrinsic GTPase activity of Gs and the H and C mutations lead to loss of, or impairment of, the GTPase activity. The intrinsic GTPase activity is necessary to terminate the interaction between Gs and adenylate cyclase, and to terminate the cAMP-mediated signaling of the given GPCR pathway. Thus, these activating mutations lead to ligand-independent activation of GPCR pathways. The tissue phenotype is determined by the role of Gs in the function of a given cell, and excess Gs/cAMP signaling in that cell. For example, pituitary somatotrophs overproduce growth hormone in a growth hormone releasing hormone-independent fashion, and gigantism/acromegaly ensues. Skeletal stem cells in the bone marrow behave as if they are under constant parathyroid hormone stimulation and fail to differentiate into osteoblasts and osteocytes, and proliferate as immature osteogenic cells and lead to the marrow fibrosis of fibrous dysplasia of bone.

The goal of this project is to identify molecules with inhibitory activity at gsp mutations that will not only allow for in-depth study of the underlying molecular and cellular pathophysiology, but also serve as compounds from which drugs to treat FD/MAS can be developed

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: IRP-FT-DE-2011-01
Assay Submitter (PI): Michael Collins, NIDCR

c. Muscular Distrophy

i. CGA

Description

Integrins are a diverse family of transmembrane cell surface receptors that mediate interactions between cells and the extracellular matrix. These heterodimeric proteins are composed of alpha and beta subunits and the a7b1 interacts with laminin in the extracellular matrix and actin of the cell cytoskeleton. Both chains contribute to ligand binding, but the alpha chain mediates the specificity of the interaction. The alpha7beta1 integrin is a major laminin receptor in skeletal, cardiac and vascular smooth muscle. Mutations in the alpha7 integrin gene are responsible for muscular dystrophy in both humans and mice. The alpha7beta1 integrin is also a major modifier of muscle disease progression in various muscle diseases including Duchenne muscular dystrophy, Fukuyama muscular dystrophy and merosin deficient congenital muscular dystrophy type 1A. In addition the alpha7beta1 integrin has been shown to be a major tumor suppressor gene in prostate cancer and plays a role in the progression of cardiovascular disease.

Transcriptional regulation of the alpha7 integrin gene is poorly understood and probably involves multiple transcription factors. The goal of identifying small molecules that enhance alpha7 integrin expression is two-fold: firstly, such molecules would help us gain insight into the complex transcriptional control of alpha7 integrin. It is known that alpha7 integrin expression is tightly controlled during various stages of development, disease and in response to external stimuli. The second goal of the project is to translate the above finding into a therapeutic strategy for muscular dystrophy. As increased alpha7 integrin levels have been shown to be beneficial in ameliorating muscular dystrophy in animal models, small molecules enhancing levels of this integrin are clearly useful and may be developed into therapeutic leads. Thus this project aims to discover small molecule probes that would not only help in gaining a better understanding of the complex regulatory mechanism of alpha7 integrin expression, but also lead to development of pharmacological agents to fight muscular dystrophy.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Centers Network [MLPCN]
MLPCN Grant: NS058429
Assay Submitter (PI): Dean Burkin, University of Nevada School of Medicine

d. Niemann Pick Type C (NPC)

i. NPC1

Description

Niemann Pick Type C (NPC) is a rare neurodegenerative lipidosis that is characterized by lipid storage in the endosomal/lysosomal system. Treatment modalities for this devastating disease are currently non-existent due to the severe obstacles associated with accessing the central nervous system with proteins or genes. The majority of mutations causing NPC disease are missense mutations that are distributed throughout the length of the NPC1 protein, with the most prevalent being the I1061T allele. Although NPC1 mutant proteins may be functional, they are typically trapped in the ER due to misfolding. Furthermore, over-expression of some mutant NPC1 proteins can rescue the disease phenotype, suggesting that up-regulation of the endogenous NPC1 mutant protein is a new drug treatment modality of the disorder.
We have developed and optimized a cell based luciferase reporter assay in 1536 well format for the identification of up-regulators of the mutant NPC1 promoter.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production centers Network [MLPCN]
MLPCN Grant: MH089375-01A1
Assay Submitter (PI): Yiannis A. Ioannou

ii. RAB9A

Description

NCGC Assay Overview:
Niemann Pick Type C (NPC) is a rare neurodegenerative lipidosis that is characterized by lipid storage in the endosomal/lysosomal system. Treatment modalities for this devastating disease are currently non-existent due to the severe obstacles associated with accessing the central nervous system with proteins or genes (Ioannou, 2000). We have developed a new paradigm, termed "Orphan Receptor Bypass Therapy" (ORByT), to address these disorders. This paradigm posits the existence of endogenous "suppressor" proteins whose expression can dramatically improve LSD pathogenesis. The goal is the identification/discovery of small chemical compounds that can modulate the expression of these proteins and provide a new treatment modality for these devastating disorders. One such protein whose overexpression reverses aspects of the NPC phenotype is Rab9.
We have developed and optimized a cell based luciferase reporter assay in 1536 well format for the identification of up-regulators of the Rab9 promoter.

NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production centers Network [MLPCN]
MLPCN Grant: MH089537-01
Assay Submitter (PI): Yiannis A. Ioannou

e. SMA

i. SMN2

Description

Spinal muscular atrophy (SMA) is caused by insufficient levels of the survival motor neuron protein SMN. The SMN locus on chromosome 5q13 contains two inverted copies of SMN called SMN1 and SMN2 which are 99% identical at the amino acid level. SMN1 is a fully functional protein and SMN2 skips exon 7 90% of the time. Skipping of exon 7 produces non-functional protein product. 10% of the SMN2 protein includes exon 7 and is fully functional.

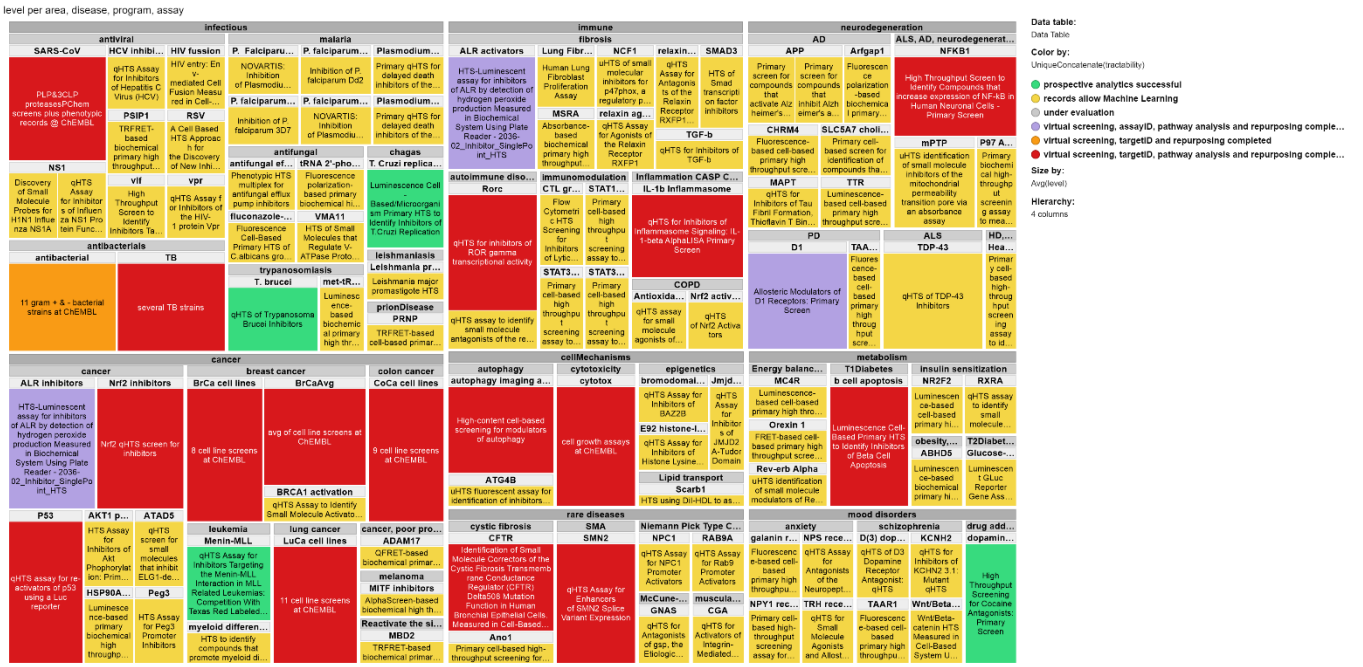
In the SMA disease state, mutations in the SMN1 locus are the cause of the disease state. Because only 10% of SMN2 is of the fully functional form, it is not sufficient to overcome the deficiency produced by the loss of the SMN1 product. A therapy that either increase the amount of SMN2 product made or to increase the inclusion of exon 7 has been proposed for the treatment of SMA.

We have designed an assay to identify small molecules that can increase the amount of functional SMN2 product by appending a luciferase reporter gene after the native SMN2 gene, such that inclusion of exon 7 in the expressed product places the luciferase sequence in frame, thus generating functional luciferase enzyme.

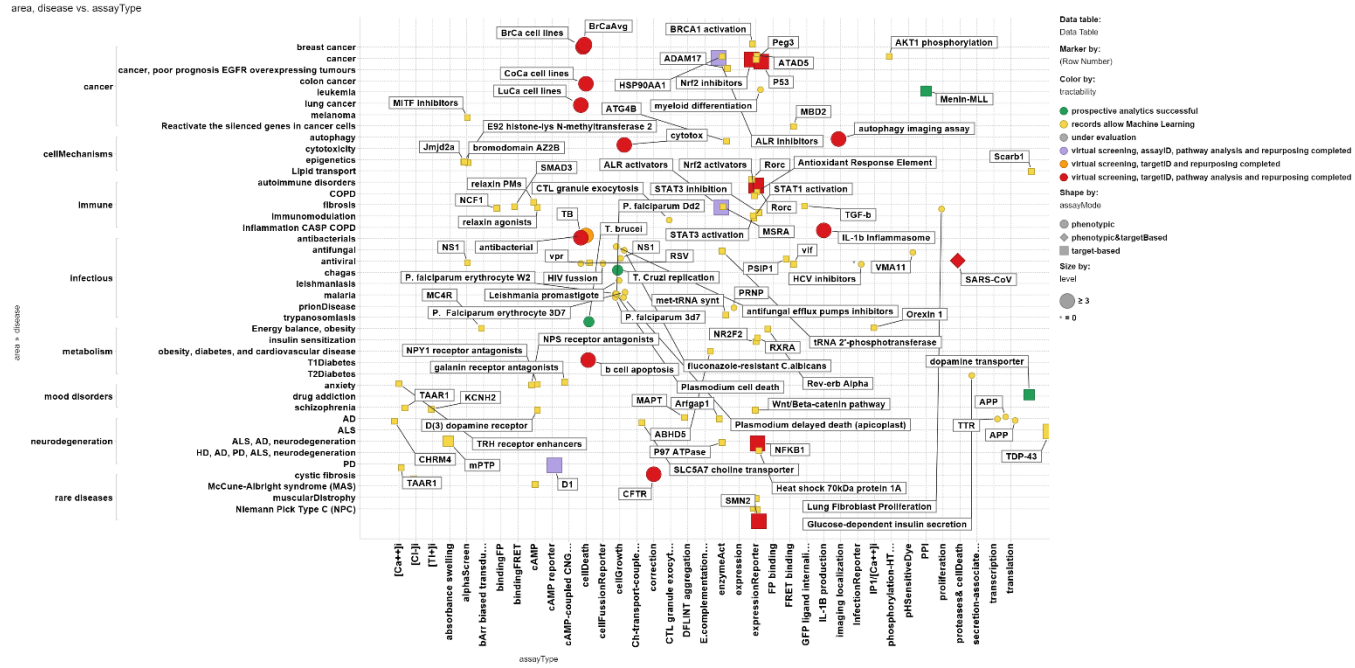
NIH Chemical Genomics Center [NCGC]
NIH Molecular Libraries Probe Production centers Network [MLPCN]
MLPCN Grant: R03 MH084179-01
Assay Submitter (PI): Elliot Androphy

Supplementary graphs

DrTargetPortfolio treemap

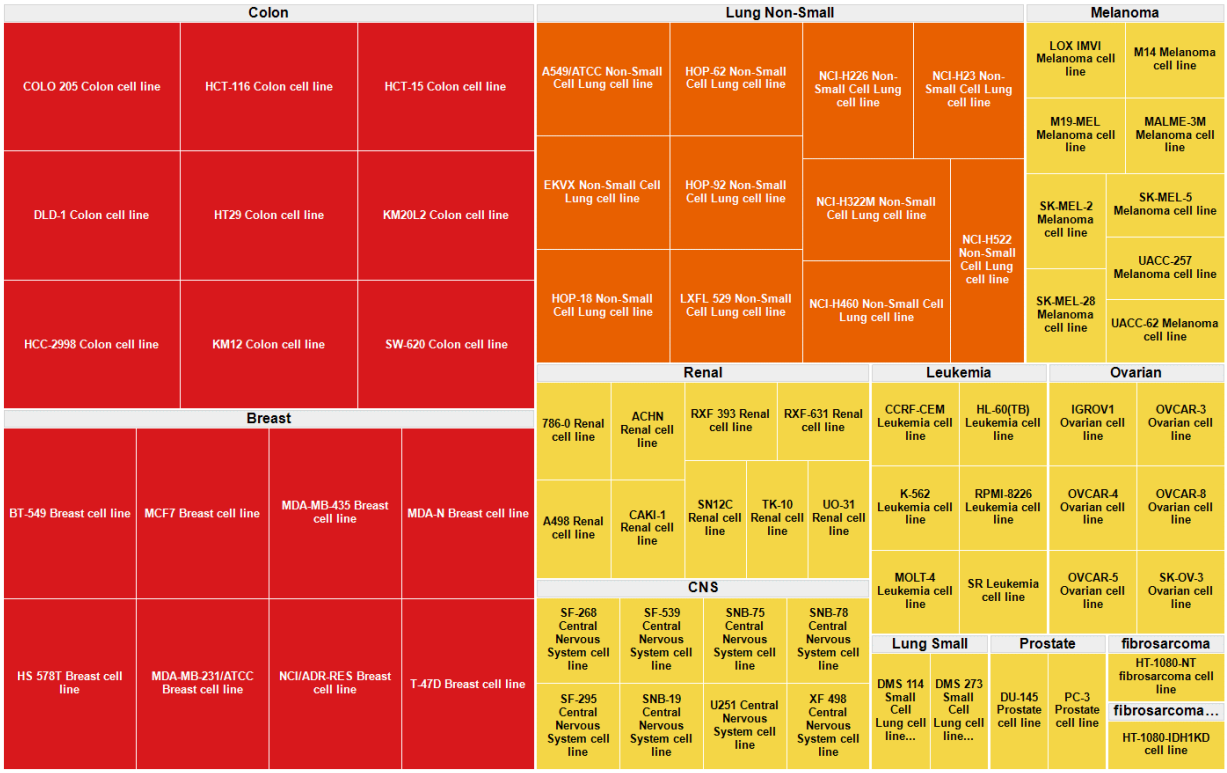


DrTarget portfolio dot plot with assay type



DrTarget tumour cell lines portfolio

level per origin, Description



Data table:
DrTargetPortfolio - Tumor Assays

Color by
UniqueConcatenate(tractability)

- prospective analytics successful
- records allow Machine Learning
- virtual screening, targetID, pathway analysis and repurposing

Size by
Avg(level)

Hierarchy
origin, Description