Drug mechanisms of action predict neurodegeneration in Alzheimer's patients

Steven Rodriguez[1,2], Petar Todorov[2], Nienke Moret[2], Bradley Hyman[1], Peter Sorger[2], Mark Albers[1,2], Artem Sokolov[2]

[1]Mass. General Institute for Neurodegenerative Disease

[2]Laboratory of Systems Pharmacology, Harvard Medical School

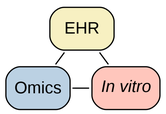
Abstract

Alzheimer's Disease (AD) is a growing epidemic as longer life expectancy fuels its principal risk factor - aging. As understanding of AD grows in the setting of many failed clinical trials, the concept of AD as a single disease is giving way to the hypothesis that it is a syndrome with multiple disease pathways progressing towards a common end-stage clinical presentation. Here, we aim to identify FDA-approved drugs that target these pathways and thus are candidates for repurposing in AD.

Given an FDA-approved drug, we asked if its mechanism of action is related to AD biology by training a predictor of disease stage. The predictor was limited to using expression of genes known to be associated with the drug, and its performance was compared to predictors constructed on randomly-selected gene sets of equal size. Thirty top-performing drugs were subsequently profiled on human neuroprogenitor cell lines that differentiate into a mixed culture of neurons, glia and oligodendroctyes to further refine their mechanisms of action in relevant cell types. Jak inhibitors Tofacitinib and Ruxolitinib were among the top performers, and additional in vitro experiments demonstrated that the two drugs can rescue inflammatory-induced neuronal death, suggesting their potential as repurposing candidates for AD.

Introduction

Joint effort



The work presented here is a part of the joint effort to identify FDA-approved drugs that can be repurposed for Alzheimer's Disease. The joint effort spans three distinct approaches to the problem: 1) conducting *in silico* drug trials using electronic health records, 2) identifying correlations between molecular mechanisms of drugs and the disease, and 3) *in vitro* experiments to assess drugs' capacity to rescue neurodegeneration and define changes to the proteome of CNS cells by the candidate drug. Each approach systematically **generates** hypotheses about potential repurposing candidates, while also **validating** hypotheses generated by its two counterparts. This poster focuses on approaches 2) and 3).

Data overview

At the histological level, AD is characterized by two main types of lesions: beta-amyloid plaques and neurofibrillary tangles[1]. The tangles arise from abnormal phosphorylation of tau, a protein thought to stabilize microtubules in neurons of the central nervous system. The distribution of tangles through the brain is used by pathologists to diagnose disease stage, known as the Braak score, in individual patients.

Braak	#Samples	Category	Pathology
0	7	A	
1	51		
2	54		
3	176	B	
4	210		
5	133	C	
6	7		

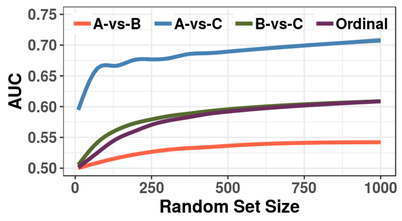
To identify associations between drug mechanisms of action and neurodegeneration, we used The Religious Orders Study and Memory and Aging Project (ROSMAP) dataset[2,3], which is available through the AMP-AD knowledge portal (https://www.synapse.org/ampad). Samples in the dataset were grouped by their Braak score, as shown in the table, to establish a three-class prediction task that distinguishes between early (A), intermediate (B), and late (C) stages of AD.

Prediction of disease stage from RNAseq data

We trained predictors of disease stage category (A, B or C) using RNAseq expression data collected on the same set of samples. Rather than using full transcriptional profiles, we reduced the gene space to sets known to be associated with specific FDA-approved drugs of interest. By comparing predictors trained on drug-related gene sets against those trained on randomly-selected sets, we generated hypotheses about drugs' relevance to neurodegeneration.

Computational setup

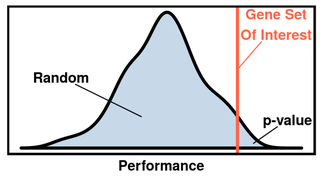
Predicting disease stage with randomly-selected gene sets



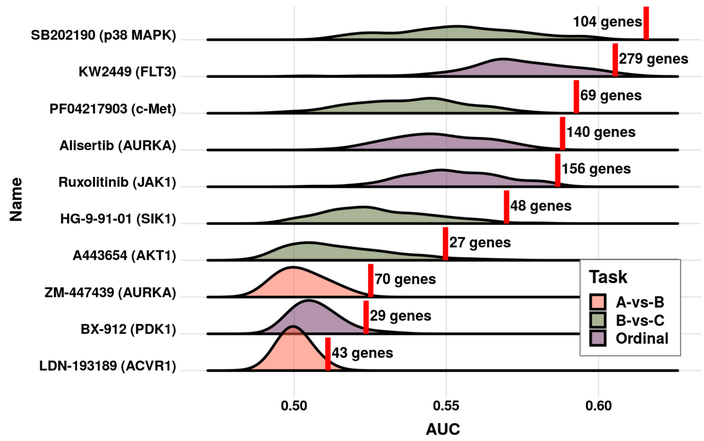
We investigated how well randomly-selected gene sets are able to predict disease stage by comparing all binary combinations of A, B, C labels, as well as considering ordinal regression. The figure shows how the performance changes with the size of gene sets and task definition. As expected, recognizing early (A) vs. late(C) disease stages is an easier prediction task than the other formulations.

Gene set as a unit of biological knowledge

Given a gene set of interest, we ask how well a predictor trained on the expression of those genes is able to predict disease stage compared to predictors trained on randomly-selected sets of the same size. If the gene set of interest leads to significantly higher performance than random sets, then it suggests association between those genes and disease.



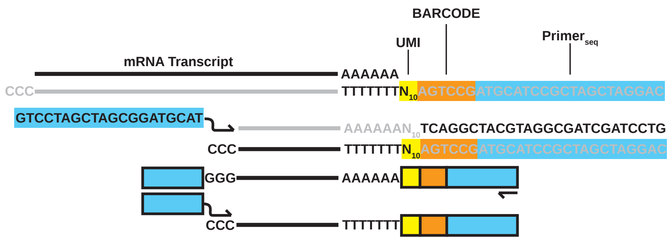
Top candidates



We composed a map of drug-gene associations by mining publicly-available resources KINOMEscan (http://lincs.hms.harvard.edu/kinomescan/), DrugBank[4] and ChEMBL[5]. The resulting gene sets were compared against randomly-selected sets of equal size, and the results for the top ten candidate drugs (with p-adj < 0.05) are presented in the figure.

Digital Gene Expression

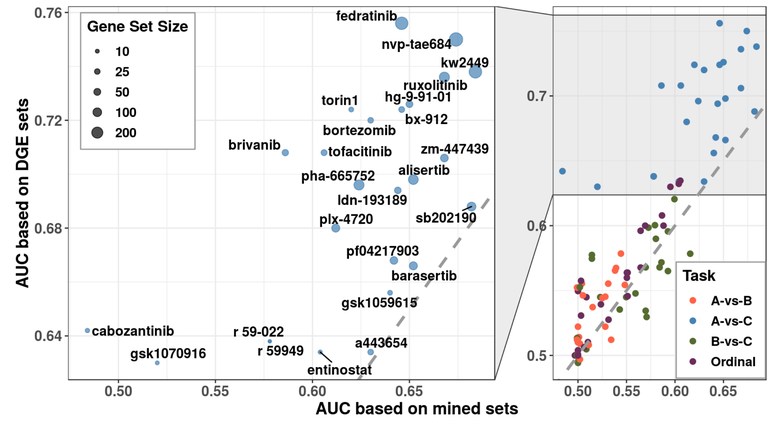
High throughput profiling



To refine drugs' mechanisms of action in relevant cell types, we profiled top 30 candidates on human neuroprogenitor cell lines that differentiate into a mixed culture of neurons, glia and oligodendroctyes. Cells treated with each drug and the corresponding controls were sequenced using the Digital Gene Expression (DGE) platform[6,7].

Experimental results

Improved prediction accuracy



Differential expression signatures were derived by comparing transcriptional profiles of treated cells against controls. By looking at the top differentially-expressed genes, we observed improved prediction accuracy over mined gene sets, particularly when learning to distinguish early (A) vs. late (C) disease stages (See Figure).

Rescue of neuronal death

Several Janus Kinase (Jak) inhibitors appeared among the top performers. We measured their ability to rescue neurodegeneration by stressing differentiated neural progenitors with poly(I:C) to induce inflammatory response and cell death. Jak inhibitors Tofacitinib and Ruxolitinib were then introduced at 10 μM concentration, leading to increased cell viability (fold change > 3, p-value < 1e-5 for both drugs).

Acknowledgments

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