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Title: Large-scale human-yeast genetic interaction for construction of disease network: systematic discovery of multiple drug targets

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Keywords: disease network; genetic interaction; yeast; drug target; amyotrophic lateral sclerosis

Corresponding Author: Kyoungho Suk

Authors: Kyoungho Suk[1,*]

Institution: [1]Department of Pharmacology, Brain Science and Engineering Institute, and Department of Biomedical Sciences, BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, Daegu, Korea,

Large-scale human-yeast genetic interaction for construction of disease network: systematic discovery of multiple drug targets

Kyoungho Suk

Department of Pharmacology, Brain Science and Engineering Institute, and Department of Biomedical Sciences, BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, Daegu, Korea

Running title: Human-yeast genetic interaction for disease interactome

Keywords: disease network; genetic interaction; yeast; drug target; amyotrophic lateral sclerosis

Abbreviations: OMIM, Online Mendelian Inheritance in Man; ALS, amyotrophic lateral sclerosis; OPTN, optineurin; ANG, angiogenin; ORF, open reading frame

Perspective to: Jo *et al.* (2017) Yeast genetic interaction screen of human genes associated with amyotrophic lateral sclerosis: identification of MAP2K5 kinase as a potential drug target. *Genome Research*. doi: 10.1101/gr.211649.116. [Epub ahead of print]

Abstract: A novel approach has been used to identify functional interactions relevant to human disease. Using high-throughput human-yeast genetic interaction screens, a first draft of disease interactome was obtained. This was achieved by firstly searching for candidate human disease genes that confer toxicity in yeast, and secondly identifying modulators of this toxicity. The study found potentially disease-relevant interactions by analyzing the network of functional interactions and focusing on genes implicated in amyotrophic lateral sclerosis (ALS), for instance. In the subsequent proof-of-concept study focused on ALS, similar functional relationships between a specific kinase and ALS-associated genes were observed in mammalian cells and zebrafish, thereby supporting the findings in the human-yeast genetic interaction screens. Finally, results of combined analyses highlighted MAP2K5 kinase as a potential therapeutic target in ALS.

Genetic mutations have been linked to a variety of human diseases. In an attempt to understand how these genes contribute to disease, a reductionist approach is often employed; a mutation in gene X causes dysfunction in protein X thereby affecting pathway X to cause

disease. Unfortunately, however, complex biological processes are far from linear and likely involve several layers of interacting networks. The yeast system provides a unique opportunity to study these interactions given the yeast genome is well characterized and amenable to genetic manipulation. Moreover, *Saccharomyces cerevisiae* yeast deletion strains tagged with a unique DNA sequence (molecular barcode) are readily available and barcode analysis by sequencing (Bar-seq) enables thousands of deletion mutants to be screened simultaneously. Although the molecular and cellular mechanisms of neurodegenerative diseases have been studied in the yeast system, the human-yeast genetic interactions were previously performed using an array format, which is laborious and time-consuming. Thus, in this study, a novel approach has been used to optimize the method for more efficient identification of genome-wide human-yeast genetic interactions for 20 Online Mendelian Inheritance in Man (OMIM) genes using a pooled and multiplex format. As a proof-of-concept, two genes associated with amyotrophic lateral sclerosis (ALS) (optineurin, OPTN; angiogenin, ANG) were subjected to further investigation. The acquired data from the two ALS-associated genes indicated that the findings in human-yeast genetic interaction were recapitulated in a mammalian cell system and zebrafish model, thereby demonstrating that this approach may ultimately lead to the identification of new therapeutic targets.

Large-scale human-yeast genetic screen: Using the OMIM gene database, Jo *et al*. identified 1,305 genes associated with human disease and cloned each individual open reading frame (ORF) into a vector placed under the control of a galactose-inducible promoter and transformed into yeast. Twenty OMIM genes were found to induce protein aggregates and toxicity in a spot assay. To investigate human-yeast genetic interactions, each of the 20 OMIM genes was introduced into yeast deletion pools (4,653 different strains) containing unique barcode sequences. All strains were pooled and individual yeast strain growth rates were quantified by barcode counting following PCR. Based on a corrected z-score, this lead to the identification of yeast toxicity modifiers that were grouped as enhancers, suppressors or no effect. When this data was validated against spot assays (10% of randomly selected modifying genes tested), average consistency for the three OMIM genes (OPTN, ANG, and CLINT1) was 77.9% for toxicity suppressors. A low degree of agreement was observed for toxicity enhancers (average consistency 24.4%). Thus, the validity of the genome-wide genetic interaction screen using toxicity modification and Bar-seq seems limited to toxicity

suppressors.

Proof-of-concept experiments: The remainder of the study focuses on genome-wide interaction data collected from two of the 20 OMIM genes identified in the initial screen, OPTN and ANG. Mutations in OPTN and ANG have both been previously linked to ALS. From the initial human-yeast genetic interaction screen, 638 OPTN and 465 ANG toxicity suppressors were identified. Human orthologs were identified for twelve of these suppressors (7 for OPTN; 5 for ANG) and the genetic interaction networks were constructed accordingly. While there was no overlap between the ANG and OPTN human ortholog toxicity suppressors, four genes (CKB2, YAP1801, MDE1, and MKK1) were found to suppress both ANG- and OPTN-induced toxicity when deleted, indicating a functional connection between the two. Jo *et al.* ultimately focused on MAP2K5 kinase (human ortholog of yeast MKK1) to determine whether a connection could be observed in a mammalian cell system. MAP2K5 inhibition by BIX 02188 compound decreased the amount of insoluble OPTN and ANG aggregates for both wild-type and disease-linked variants in transfected NIH3T3 cells. BIX treatment, however, did not alter the expression of ANG or OPTN. In addition to using mammalian cells, overexpression of ANG or OPTN mutants caused motor axonopathy in the spinal cord of zebrafish embryos, and that morpholino-induced knockdown of MAP2K5 rescued mutant OPTN- and ANG-induced motor axonopathy. These data indicate that MAP2K5 has a disease modifying function in the mutant OPTN- and ANG-induced zebrafish models of ALS. Moreover, MAP2K5 inhibition enhanced autophagy flux, implicating autophagy in the protective mechanisms of MAP2K5 in ALS.

In conclusion, to better understand disease pathways, a human-yeast genetic interaction screen for human disease genes was performed in a pool format. For human disease genes with yeast toxicity, a genetic interaction screen was performed using a library of yeast deletion mutants. Genetic interactions that reduced toxicity were identified with multiplexed barcode sequencing. Subsequent studies focused on ALS-associated genes, their toxicity modifiers, and network analysis indicated that the human orthologs of the yeast toxicity modifiers of the ALS genes were involved in cell death, lipid metabolism, and molecular transport. Further investigation in mammalian cells and zebrafish identified MAP2K5 as a potential therapeutic target for ALS.

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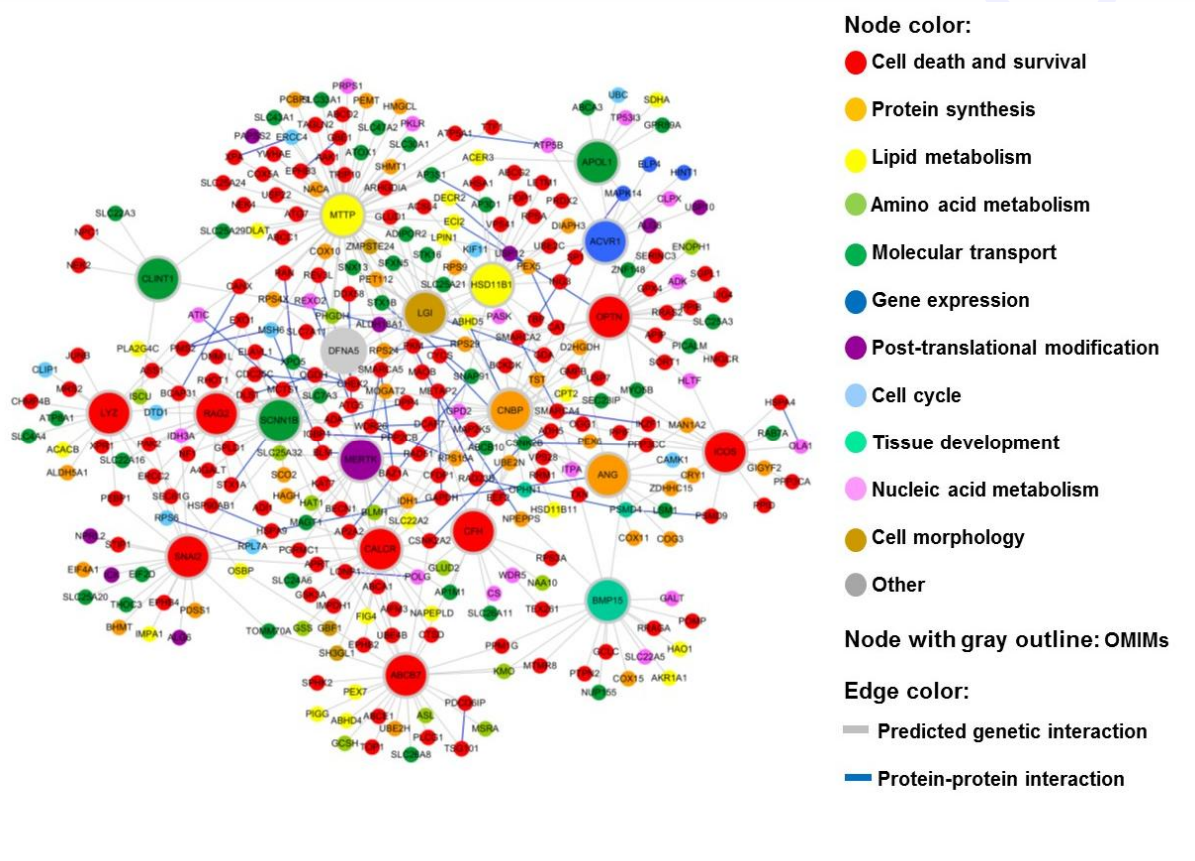
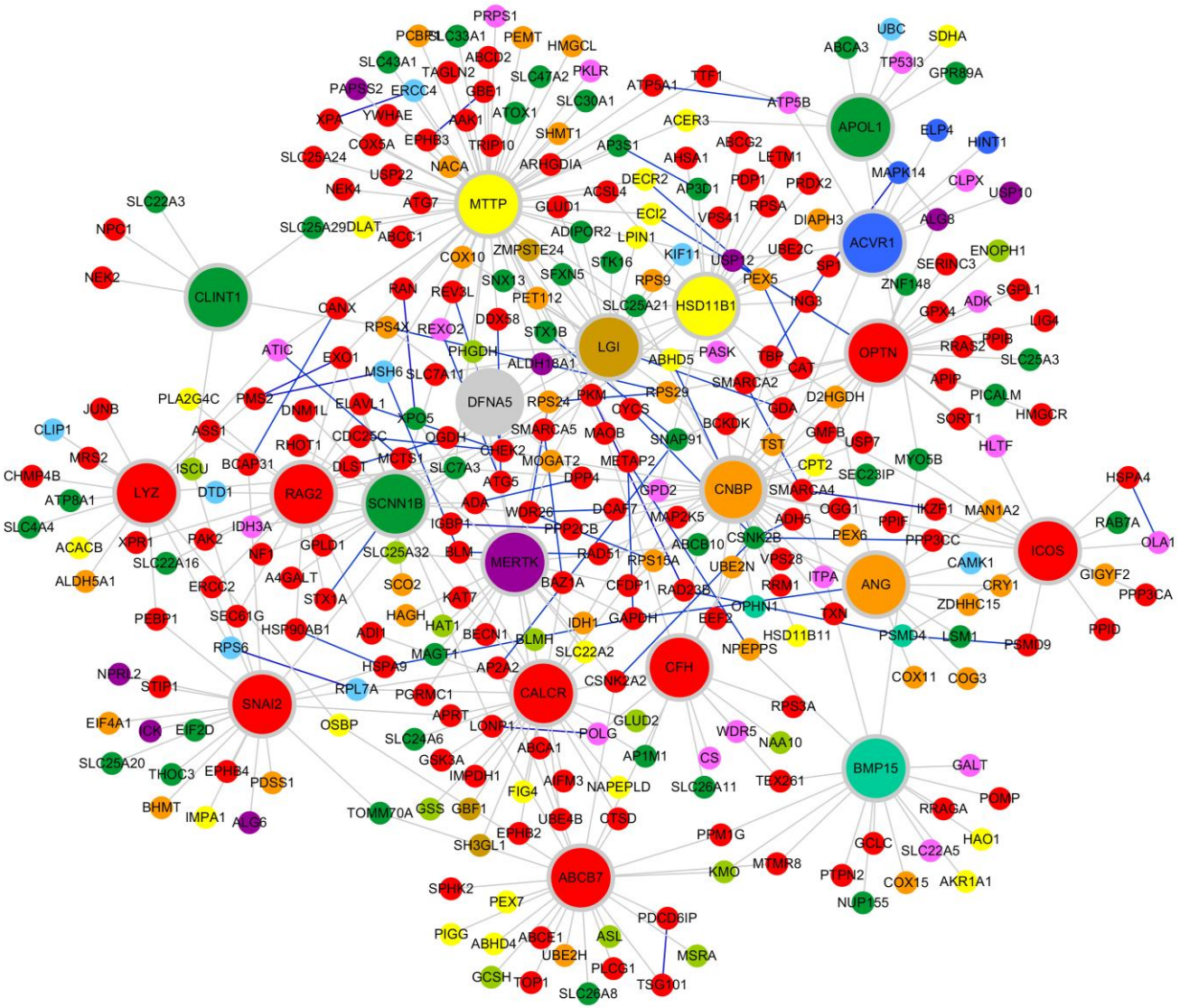


Figure 1. Disease interactome based on human-yeast genetic interaction screens. Human orthologs of yeast genes whose deletion suppressed the toxicity of the 20 OMIM ORFs were identified. A network view of these human orthologs was generated using Cytoscape. The node color corresponds to the biological function category to which the gene belongs. The color of an edge indicates the type of interaction. Adapted from Jo *et al.*, *Genome Res* (2017).



Node color:

- Cell death and survival
- Protein synthesis
- Lipid metabolism
- Amino acid metabolism
- Molecular transport
- Gene expression
- Post-translational modification
- Cell cycle
- Tissue development
- Nucleic acid metabolism
- Cell morphology
- Other

Node with gray outline: OMIMs

Edge color:

- Predicted genetic interaction
- Protein-protein interaction