Perspective

Human-yeast genetic interaction for disease network:
systematic discovery of multiple drug targets

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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 first searching for candidate human disease genes that confer toxicity in yeast, and second, identifying modulators of toxicity. This study found potentially disease-relevant interactions by analyzing the network of functional interactions and focusing on genes implicated in amyotrophic lateral sclerosis (ALS), for example. 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, supporting findings in human-yeast genetic interaction screens. Results of combined analyses highlighted MAP2K5 kinase as a potential therapeutic target in ALS. [BMB Reports: Perspective 2017; 50(11): 535-536]

Genetic mutations are linked to a variety of human diseases. To understand how these genes contribute to disease, a reductionist approach is often used; a mutation in gene X causes dysfunction in protein X affecting pathway X to cause disease. Unfortunately, complex biological processes are far from linear and likely involve several layers of interacting networks. The yeast system provides a unique opportunity to study interactions given yeast genome is well characterized

and amenable to genetic manipulation. *Saccharomyces cerevisiae* yeast deletion strains tagged with 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 molecular and cellular mechanisms of neurodegenerative diseases have been studied in the yeast system, human-yeast genetic interactions were previously conducted using an array format, that is laborious and time-consuming. In this study, a novel approach was 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 pooled and multiplex format (Diagram 1). As a proof-of-concept, two genes associated with amyotrophic lateral sclerosis (ALS) (optineurin, OPTN; angiogenin, ANG) were subjected to further investigation. Acquired data from the two ALS-associated genes indicated that findings in human-yeast genetic interaction were recapitulated in a mammalian cell system and zebrafish model, validating that this approach may lead to 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 control of a galactose-inducible promoter and transformed into yeast. Twenty OMIM genes induced 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. Strains were pooled and individual yeast strain growth rates were quantified by barcode counting following PCR. Based on corrected z-score, this led to identification of yeast toxicity modifiers grouped as enhancers, suppressors or no effect. When this data was validated against spot assays (10 percent of randomly selected modifying genes tested), average consistency for the three OMIM genes (OPTN, ANG, and CLINT1) was 77.9 percent for toxicity suppressors. A low degree of agreement was observed for toxicity enhancers (average consistency 24.4 percent). Validity of genome-wide genetic interaction screen using toxicity modification and Bar-seq is limited to toxicity suppressors.

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Diagram 1. Disease interactome based on human-yeast genetic interaction screens. Human orthologs of yeast genes of which deletion suppressed toxicity of the 20 OMIM ORFs were identified. A network view of 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 type of interaction. Adapted from Jo, et al., *Genome Res* (2017).

Proof-of-concept experiments: The remainder of this 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 are 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 12 of these suppressors (7 for OPTN; 5 for ANG) and 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) suppressed ANG- and OPTN-induced toxicity when deleted, indicating a functional connection between the two. Jo, et al. focused on MAP2K5 kinase (human ortholog of yeast MKK1) to determine if a connection may be observed in a mammalian cell system. MAP2K5 inhibition by BIX 02188 compound decreased the amount of insoluble OPTN and ANG aggregates for wild-type and disease-linked variants in transfected NIH3T3 cells. BIX treatment did not alter 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 morpholino-induced knockdown of MAP2K5 rescued mutant OPTN- and ANG-induced motor axonopathy. Data indicate that MAP2K5 has a disease modifying

function in mutant OPTN- and ANG-induced zebrafish models of ALS. MAP2K5 inhibition enhanced autophagy flux, implicating autophagy in protective mechanisms of MAP2K5 in ALS.

In conclusion, to better understand disease pathways, a human-yeast genetic interaction screen for human disease genes was conducted in pool format. For human disease genes with yeast toxicity, a genetic interaction screen was conducted 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 indicate that human orthologs of yeast toxicity modifiers of ALS genes were involved in cell death, lipid metabolism and molecular transport. Further investigation of mammalian cells and zebrafish identified MAP2K5 as a potential therapeutic target for ALS.

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