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The Synthetic Genetic Interaction Network Reveals Small Molecules that Target Specific Pathways in *Saccharomyces cerevisiae*

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Abstract

High-throughput elucidation of synthetic genetic interactions (SGIs) has contributed to a systems-level understanding of genetic robustness and fault-tolerance encoded in the genome. Pathway targets of various compounds have been predicted by comparing chemical-genetic synthetic interactions to a network of SGIs. We demonstrate that the SGI network can also be used in a powerful reverse pathway-to-drug approach for identifying compounds that target specific pathways of interest. Using the SGI network, the method identifies an indicator gene that may serve as a good candidate for screening a library of compounds. The indicator gene is selected so that compounds found to produce sensitivity in mutants deleted for the indicator gene are likely to abrogate the target pathway. We tested the utility of the SGI network for pathway-to-drug discovery using the DNA damage checkpoint as the target pathway. An analysis of the compendium of synthetic lethal interactions in yeast showed that superoxide dismutase 1 (*SOD1*) has significant SGI connectivity with a large subset of DNA damage checkpoint and repair (DDCR) genes in *Saccharomyces cerevisiae*, and minimal SGIs with non-DDCR genes. We screened a *sod1* strain against three National Cancer Institute (NCI) compound libraries using a soft agar high-throughput halo assay. Fifteen compounds out of ~3100 screened showed selective toxicity toward *sod1* relative to the isogenic wild type (wt) strain. One of these, **1A08**, caused a transient increase in growth in the presence of sublethal doses of DNA damaging agents, suggesting that **1A08** inhibits DDCR signaling in yeast. Genome-wide screening of **1A08** against

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Supplemental Information. Detailed description of method used for selection of deletion mutant; ¹H NMR spectrum of pure **1A08**; Table showing SGIs between all yeast genes and DDCR pathway.

the library of viable homozygous deletion mutants further supported DDCR as the relevant targeted pathway of **1A08**. When assayed in human HCT-116 colorectal cancer cells, **1A08** caused DNA-damage resistant DNA synthesis and blocked the DNA-damage checkpoint selectively in S-phase.

Introduction

Two genes are said to interact synthetically if the double mutant resulting from the deletion of both genes is either more or less fit than expected based on the fitness of the single mutants. While there are many types of SGIs, for simplicity we use the term SGI to refer only to gene deletion pairs resulting in significant fitness loss or lethality not expected from their single deletion mutants. Genes related by synthetic lethality tend to operate in parallel or mutually compensating pathways, and systematic genome-wide synthetic lethality screens have revealed functional relationships between biological pathways[1–2].

For example, genes with *congruent*, or highly similar, SGI connections often participate in common cellular pathways. This observation has led to the chemical analog of synthetic lethality, chemical-genetic interaction (CGI) in which a drug causes lethality only in the context of a genetic deletion. Patterns of CGIs have been used to fingerprint small molecules and to identify their modes of action. For example, CGIs have been used to identify compounds that induce DNA damage by screening for selective toxicity in deletion strains that were defective in DNA repair pathways[3]. Genome-wide drug sensitivity screens have yielded insights into drug mechanisms of action[4–6] and their biomolecular targets[7–11].

Chemical-genetic maps have been established, showing that compounds with similar mechanisms produce similar chemical-genetic sensitivity profiles[5,4]. By clustering chemical-genetic profiles together with genetic-synthetic profiles from the SGI network, compound modes-of-action can often be predicted by clustering compounds with genes of known functions. These results demonstrate that the SGI network can be used to make inferences in the drug-to-pathway direction; i.e. genetic-synthetic interactions can be used to identify a potential pathway target of a given compound. However, an open question remains: can the SGI network also be used in reverse? In other words, can the SGI network be used to identify small molecules that disrupt a particular target pathway of interest?

The reverse pathway-to-drug problem has high practical benefit as it would allow us to conduct focused searches for compounds that target clinically relevant pathways of interest. For example, identifying new anti-cancer therapeutics based on newly identified cancer-specific processes would increase the repertoire of agents to battle tumorigenesis. As another example, identifying antibiotics that target microbe-specific pathways not targeted by any currently available compounds would provide another defense against drug-resistant strains. Thus, rather than predicting pathway targets of large chemical libraries using the forward drug-to-pathway approach, the pathway-to-drug approach would offer an efficient means by which to identify compounds of interest.

The goal of the reverse pathway-to-drug approach is to find a compound with bioactivity against a pathway of interest. From the pathway's perspective, we refer to such a compound

as a *hit compound*. Current high-throughput screening (HTS) technology allows cells harboring a deletion in an indicator gene to be screened against a large library of compounds[12]. Compounds that show differential lethality toward the mutant relative to wild type are selected as hits and then moved into secondary screens to test their effect on the pathway of interest. We refer to genes with mutants that produce sensitivities highly specific for a pathway as *indicator genes* for that pathway.

We provide evidence that the SGI network conveys key information for addressing the reverse pathway-to-drug problem. Specifically, SGI patterns reveal which genes are informative for use in a mutant-based chemical screen to identify small molecules that target a pathway of interest. For a given pathway, some genes are *pathway hubs* (*p-hubs*) which exhibit SGI connections specific to that pathway. These p-hubs have more synthetic interactions to members of the given pathway, compared to outside of the pathway, than expected by chance (Figure 1). In order to test whether p-hubs serve as good indicators for screening chemical libraries, we tested several drugs that have known targets and found that the current SGI network indeed provided indicator genes for several pathways.

We performed a follow-up screen using an indicator gene predicted by the SGI network for the DNA damage checkpoint and repair (DDCR) pathway. The indicator gene with the most specific set of SGIs to the DDCR pathway was the superoxide dismutase gene, *SOD1*. We screened three chemical libraries from the National Cancer Institute (NCI) for compounds that killed the *sod1* deletion (*sod1*⁻) strain but had relatively little effect on wild type (wt). Fifteen compounds showed differential activity. Follow-up studies on one of these compounds, **1A08**, revealed that it targets the DDCR pathway in yeast and disrupts the intra-S-phase cell cycle checkpoint in human HCT-116 cells. Thus, the SGI network successfully predicted *SOD1* can be used as an indicator gene to screen for DDCR pathway inhibitors.

Materials and Methods

Pathway Genetic Interaction Score

Pathway hubs (p-hubs) have a high fraction of synthetic genetic interaction (SGI) links to a set of genes in a target pathway while having few SGIs to genes outside of the target pathway. To quantify this specificity, we used a measure that reflects the statistical significance of observing a certain fraction of links in common with members of the target pathway. Genes that have a large number of SGI neighbors will also have more genes in common with the target pathway by chance. Therefore, we take into account the total set of neighbors by using a score based on the hypergeometric distribution. Let n_g be the number of genes that are SGI neighbors of gene g , m_t be the number of genes known to participate in target pathway t , and q_{gt} be the number of SGI neighbors of g in pathway t . The probability that g 's SGI neighborhood shares q_{gt} or more genes with the pathway by chance is given by the hypergeometric distribution:

$$P(X \geq q_{gt}) = \sum_{x=q_{gt}}^{n_g} \frac{\binom{m_t}{x} \cdot \binom{N-m_t}{n_g-x}}{\binom{N}{n_g}},$$

where N is the total number of unique genes in the SGI network (i.e. $N=2702$). Note that x in the summation takes on all possible overlaps that are equal to or greater than the observed amount q_{gt} , which calculates the upper tail of the cumulative probability distribution. This quantity represents the p -value of observing q_{gt} genes in common; small probabilities equal higher significance. We define the *pathway genetic interaction (PGI) score* as the negative logarithm of the above to measure the degree to which gene g 's SGIs specifically connect to members of pathway t :

$$PGI(g, t) = -\log_{10} P(X \geq q_{gt}).$$

The score provides a guideline for the significance of a gene's SGI overlap. For example, a score equal to 3 can be interpreted as a significance level associated with a P value equal to 0.001.

Differential Sensitivity

For drugs with known pathway targets, we quantify the degree to which an indicator's deletion mutant is more sensitive to hit than non-hit compounds using the publicly available chemical-genomics compendium from Hillenmeyer, et al.[6]. Let S represent the chemical-genomics compendium encoded as an n -by- r matrix of n genes and r genome-wide sensitivity measurements for d drugs ($d \leq r$). In addition, let D represent the set of drugs included in the compendium. For a particular pathway t , let D_{t+} be the set of hitting compounds and D_{t-} be the set of drugs known to target other pathways. Note that D_{t-} excludes drugs of unknown mechanism because there is a chance that such drugs do indeed target the pathway of interest. Because sensitivity can vary considerably across experiments in the compendium, we use a robust method to quantify the degree to which an indicator is sensitive to hit relative to non-hit compounds. We use a *difference in sensitivity* score computed from a mutant's sensitivity data:

$$DSS(g, k) = \frac{m(g, D_{k+}) - m(g, D_{k-})}{s(g, D)},$$

where $m(i, A)$ is the median sensitivity of the mutant to drugs in set A , $m(i, A) = \text{median}\{S_{ij}\}_{j \in A}$, and $s(i, A)$ is the median absolute deviation of the mutant's sensitivities to drugs in set A , i.e. $s(i, A) = \text{median}\{|S_{ij} - m(i, A)|\}_{j \in A}$. The DSS score reflects the average tendency for a mutant to be sensitive to hit compared to non-hit compounds.

Cell lines

The HCT-116 cell lines were a gift of Dr. B. Vogelstein. Cells were grown in McCoy's 5A media supplemented with 10% FBS, penicillin, and streptomycin at 37°C in a humidified environment with 5% CO₂. Plating of cells into 384-well plates was performed using a Thermo Multidrop 384. Both *sod1* and wt yeast strains were in the BY4743 background[13].

Chemicals

CPT (Sigma) stock solutions of 100 µM were stored in DMSO, 10 mM BrdU (Sigma) stock solutions were stored in PBS, and a 20 mM caffeine (Acros) stock solution was stored in McCoy's 5A media. All solutions were stored at -20°C until use. The chemical libraries were obtained from the National Cancer Institute as plated DMSO stocks (1 mM or 10 mM). All 3104 compounds screened were from the NCI Diversity, Mechanistic, and Natural Products libraries (http://dtp.nci.nih.gov/branches/dscb/repo_open.html). The compounds were formatted into 384-well plates as 1 or 10 mM DMSO stock solutions.

Soft Agar Assay

We tested both wild type and *sod1* yeast using the HT yeast halo assay reported previously[14]. A 300 µL suspension of saturated yeast culture was added to 25 mL YPD containing 0.75% agar at 50°C. 20 mL of this solution was added to omni trays (Nunc) and allowed to solidify. A 384 pin array tool was used to transfer the NCI compound libraries and the yeast were allowed to grow for 24 h. The plates were then read in a standard plate reader at OD₆₀₀.

Genome-wide profiling of 1A08 in barcoded yeast library

The homozygous yeast deletion pool was constructed as described[15] and stored in 20 µL aliquots at -80°C. Aliquots of the pool were thawed and diluted in YPD to an optical density at 600 nm (OD₆₀₀) of 0.0625 and a final volume of 700 µL. **1A08** (or DMSO) was then added and the pool was grown for five generations in a Tecan GENios microplate reader, at which point cells were robotically saved to a 4°C cooling plate. Genomic DNA was then purified and used as a template for the TAG PCR reactions[15]. Biotinylated PCR products were hybridized to Genflex TAG3 arrays (Affymetrix) for 16 h, washed, labelled with streptavidin-conjugated phycoerythrin, and scanned with a GeneChip 3000 Scanner (Affymetrix). Raw intensity values were mean normalized and sensitivity values calculated as described in reference[6].

Flow Cytometry

HCT-116 cells were trypsinized, washed with PBS, and fixed with 4% formaldehyde in PBS for 15 min at room temperature. They were then washed once with PBS and incubated at 37°C with PBS plus 100 µg of RNase A for 4 hours. The cells were pelleted and resuspended in 50 µg/mL propidium iodide / 0.05% Tween 20 in PBS. The tubes were put on ice and read with a BD Biosciences FACSCalibur.

BrdU incorporation assay

HCT-116 cells were seeded at a density of 10,000 cells per well in a 96-well white/opaque plate (Costar) and were allowed to adhere overnight. Wells were treated with 50 nM CPT and 10 μ M BrdU, plus 2 mM caffeine or various concentrations of **1A08**. The cells were incubated for 24 h at 37°C and then analyzed as follows. Media was removed and the plates were washed once with TBS. A 50 μ L ice-cold 70% EtOH / 30% PBS solution was used to fix the cells overnight at 4°C. The EtOH / PBS mixture was removed and the plates were rinsed once with TBS. 50 μ L of a 2M HCl / 0.5% Tween 20 solution was added for 20 min. The plates were then washed in succession with 150 μ L of 10% 2M NaOH / 90% Hank's Buffered Saline Solution (HBSS) and then twice with 150 μ L of HBSS. A blocking solution (75 μ L) consisting of TBS / 2.5% Milk / 0.1% Tween 20 (TBSMT) was added for 45 min. This was removed and 50 μ L of TBSMT / 0.5 μ g/mL of a mouse anti-BrdU antibody / 1:2000 dilution of an anti-Mouse Ig-HRP conjugated antibody (Santa Cruz Biotech) was added for 90 min. The plates were then washed using TBS (3x). 20 μ L of Pierce Super Signal was added and the plates were read using a standard plate reader in the luminescence mode.

G1 checkpoint assay

HCT-116 cells were plated in 6-well plates and allowed to adhere for 24 h. Nocodazole and 50 nM CPT were then added in combination with DMSO, **1A08**, or caffeine for 6 h before being analyzed by flow cytometry as described above.

G2 checkpoint assay

HCT-116 p53 +/+ or HCT-116 p53 -/- cells were plated in 6-well plates and allowed to adhere for 24 h. Nocodazole and 10 nM CPT were then added, in combination with DMSO, **1A08**, or caffeine for 24 h. Cells were fixed and stained with Hoescht dye, and the percent of cells in mitosis was determined by counting mitotic cells using fluorescence microscopy. Three separate sets of five random fields were chosen and counted, with the number of cells counted for each condition ranging from 147 to 196.

Results

SGI Connectivity Is Correlated With Differential Drug Sensitivity

We collected a set of drugs as controls that were profiled in the Hillenmeyer *et al.* dataset[6] and are known to inhibit the function of a specific pathway. Of these controls, twenty-one were defined as positive controls because they target specific gene products. These positive controls included two anti-metabolites, three microtubule inhibitors, three cell wall inhibitors, and thirteen other compounds targeting ten additional cellular processes (Table 1 and Table S1). We collected a large set of SGIs from the BioGRID database[16,17] and retrieved 33,487 interactions for 3715 genes (45% of the genome; see Table S2). For a given pathway, each gene was scored for its degree of synthetic genetic interaction with the given pathway (*pathway genetic interaction*, or PGI) and its relative sensitivity to drugs that are known to target that pathway (*differential sensitivity score*, or DSS) (Figure 1).

To test if a gene's SGI connectivity to a pathway predicts its usefulness as an indicator of a specific pathway, we measured the correlation between the DSS and PGI scores for these positive control drugs. Specifically, we collected genes that were either disconnected from the pathway or had moderate to high SGI connectivity based on the PGI score. Ten out of the twelve positive control pathways (83%) exhibited a positive correlation between the PGI score and the differential sensitivity to drugs that are known to target those pathways (Figure 2). For example, indicator genes with the highest PGI scores to actin-related processes are differentially sensitive to the actin poison latrunculin. As expected, both negative controls also showed little to no correlation between PGI and DSS. On the other hand, positive control pathways related to the proteasome and histone deacetylase inhibitors had lower DSS for genes with high PGI scores. This may be due to lack of completeness in the SGI network, or to a lack of correspondence between genetic deletion and small molecule inhibition for these targets. In general, we observed that high PGI scores were indicative of high differential sensitivity. This result suggests that knockouts of genes with specific SGI connectivity to a pathway of interest can be used to identify drugs that target that pathway.

A global survey of pathway hubs defined by the SGI Network

To identify p-hubs, we considered in turn each of 187 Gene Ontology (GO) categories defined by Myers *et al.* (2006) [18] and one constructed to represent the DNA damage checkpoint and repair (DDCR) pathway. Of these, 48 had at least one significant p-hub (see Table S3). A global overview of these 48 pathways and all genes scored by PGI to the pathways is shown in Figure 2. The memberships of the genes in the Myers GO categories are also shown (right hand heatmap in Figure 2). The top-scoring gene, or predicted p-hub, is shown in Table 2. The pathways contained in each of the clusters in Figure 2 are available in Table S3.

To a large extent, p-hubs were found to belong to the target pathway (*intrinsic p-hubs*), while some examples were present of p-hubs from parallel pathways (*extrinsic p-hubs*). Several clusters (A, D, F, and I) with pathways involved in DNA metabolic processes, such as DNA repair, replication, and packaging, contain primarily intrinsic p-hubs. Similarly, vesicle-mediated fusion (cluster J), along with protein localization and nuclear transport (cluster Q), is mostly associated with p-hubs belonging to the pathway. For example, NUP2, a nucleoporin, was the top p-hub for the nuclear transport target pathway (Table 2). The top scoring p-hub for DNA packaging was HTZ1, which is itself a histone variant.

While the general rule for these pathways is that p-hubs belong to the pathway, there were some examples of those with extrinsic p-hubs. For example, cluster N contained pathways related to mitosis and chromosome segregation. Several p-hubs are not listed as members of this pathway, but have roles that implicate them as connected with microtubule function such as NUM1. Interestingly, several proteins involved in the co-chaperonin prefoldin complex – GIM2, GIM3, and GIM5 – were revealed as mitotic p-hubs. The top p-hub for cytoskeleton organization and biogenesis was GIM4 (Table 2). Consistent with this is the observation that GIM2 and GIM5 deletion mutants are sensitive to the microtubule poisons nocodazole and benomyl. The top-scoring gene for DNA damage checkpoint and repair was the extrinsic p-hub superoxidized dismutase family member, SOD1. The definition of this

pathway and follow-up experiments to validate this observation are presented in detail below.

The SGI network identifies SOD1 as a pathway hub for DNA damage checkpoint and repair

Cells monitor and maintain genome integrity through a network of signaling and repair pathways that we collectively refer to as the DNA damage checkpoint and repair (DDCR) pathway. DNA damage can activate checkpoints throughout the cell cycle, with distinct responses at G1, S, and G2 leading to a pause in cell cycle progression, initiation of DNA repair, and, in some cases, initiation of apoptosis or senescence[19,20]. Checkpoints are highly conserved in evolution, and loss-of-function mutations in many checkpoint proteins are associated with genomic instability and, in higher eukaryotes, increased predisposition to cancer[21]. There is strong evidence that DDCR inhibitors such as UCN-01 can synergize with DNA damaging anticancer drugs in vivo[22], although there are relatively few drugs known to target DDCR specifically.

To identify the most informative deletion mutant to screen for DDCR inhibitors, we first identified 210 genes annotated with either DNA damage, checkpoint, or repair by their known Gene Ontology (GO) functions[23]. 897 genes had a synthetic defect interaction with at least one of the 210 genes in the DDCR pathway (Table S3). We then identified the best p-hub for DDCR based on its PGI score to that pathway. The best p-hub for DDCR is SOD1, the gene that encodes the oxygen radical detoxifying enzyme superoxide dismutase. Figure 4A shows a histogram plot of the genes that exhibit SGIs with the DDCR pathway, and their hypergeometric P-values according to the above analysis. Thus, *SOD1* has the most significant score when comparing the number of interactions between *SOD1* and the DDCR set to the number of interactions between *SOD1* and genes outside the DDCR set. Figure 4B shows the total number of genes that were labeled DDCR, with the known *SOD1* interactions comprising 27 of the DDCR genes and 13 genes outside the DDCR pathway. By comparison, the second most significant gene, *RAD27*, interacts with almost twice as many DDCR genes, but has 10 times as many non-DDCR interactions (Table S3).

The majority of the genetic interactions identified for *SOD1* are derived from a genome-wide SGI analysis of the yeast DNA integrity network by Pan *et al.*, in which 74 query genes involved in all aspects of DNA replication and DNA replication checkpoint signaling were screened for SGIs across the yeast genome[24]. *SOD1* showed SGIs with several pathways related to DNA damage checkpoint and repair signaling, including base excision repair, homologous recombination, postreplication repair, and DNA-damage and replication checkpoints.

As a scavenger of DNA-damaging superoxide radicals, it is not surprising that Sod1p is essential in mutants lacking functional DNA repair and DNA damage checkpoint mechanisms. Likewise, DDCR genes become essential in the absence of the radical scavenger Sod1p. This gene is not annotated as part of the DDCR pathway, and is therefore a good test of whether a pathway can be targeted in a small molecule screen using an extrinsic p-hub rather than simply chosen from within the pathway of interest.

Screening against *sod1*

We used the high-throughput yeast halo assay reported by us previously[14] to screen for compounds that exhibit selective toxicity toward the *sod1* deletion strain, with the aim of identifying compounds that target the DDCR pathway in yeast. Using the mechanistic, diversity, and natural products libraries from the NCI Developmental Therapeutics Program, we tested 3104 compounds for a differential growth effect between *sod1* and the parental wild-type strain. A total of 15 compounds exhibited some degree of selective lethality toward *sod1* (Figure 5). Most of these compounds contain oxidizing moieties consistent with the role of Sod1p in protecting the cell against oxidative stress[25]. For example, 8 of the hits are quinones, which are known to generate reactive oxygen species (ROS) *in vivo*[26]. Compound **NSC-307454** is a nitrosourea similar in structure to streptozotocin, a compound known to induce oxidative stress in mammalian cells via the production of nitric oxide[27]. Compound **NSC-130796** is a dimer of the antibacterial compound quinoxaline, and **NSC-207895** is a derivative of nitrobenzofuroxan, a highly electrophilic compound known to have both antitumor[28] and mutagenic[29] activities. Biguanide **NSC-401366** is a derivative of phenanthrene. Biguanides such as synthalin A and metformin have been used historically as anti-hyperglycemic drugs. Furthermore, bis-biguanide chlorhexidine (CHX) is used as an antiseptic in dentistry, and has been shown to cause an increase in lipid peroxidation mediated by Fe³⁺ and reactive oxygen. No biological activity has been reported for compounds **NSC-47621**, **NSC-57975**, or **NSC-629301**.

Secondary screening: DNA damage checkpoint abrogation in yeast

To filter potential DDCR-active compounds from those that cause oxidative stress, we performed a secondary screen to test their ability to abrogate the DNA damage checkpoint in yeast. Increased growth in the presence of DNA damaging agents is analogous to the radioresistant DNA synthesis (RDS) phenotype observed for many types of checkpoint-deficient cells, including yeast[9]. Therefore, we tested the hit compounds for their ability to accelerate growth in the presence of methyl methanesulfonate (MMS) using a disc halo assay. Of the 15 compounds, only one caused a halo of growth when spotted onto a plate containing 0.02% MMS (Figure 6A, iii). This compound, **NSC-629301**, also caused a growth halo in the presence of 1 mM cisplatin (Figure 6A, v). At lower doses of MMS and cisplatin, **NSC-629301** had a growth inhibitory effect (Figure 6A, ii and iv), also consistent with checkpoint abrogation[9]. We call this compound by its plate and well ID, **1A08**.

We next asked whether the disc halo assay could be recapitulated in liquid culture. We added either 10 μ M or 25 μ M of **1A08** to a growing yeast culture in the presence of .02% MMS. After the cultures were allowed to grow for 48 hours, **1A08** caused increased growth in the presence of 0.02% MMS over MMS alone (Figure 6B).

Genome-wide sensitivity to 1A08

To comprehensively identify genes important for conferring resistance to **1A08**, we assayed the fitness of ~4800 bar-coded homozygous deletion strains (representing virtually all non-essential genes in yeast) in the presence and absence of **1A08**. A single pooled culture was grown in 17 μ M **1A08**, and individual strain sensitivity (relative to untreated controls) was

determined as described previously[15]. We found the *sod1* strain to be the second-most sensitive strain in this experiment (Figure 7). We also found that sensitive strains were enriched for multidrug resistant (MDR) genes, such as those involved in vacuolar protein transport[6]. Similar sensitivity profiles were observed when the experiments were performed at 50 and 68 μ M **1A08**, and these sensitivities were confirmed for selected strains, including *sod1*, in isogenic cultures (data not shown). Figure 8 shows the selectivity of **1A08** toward the *sod1* deletion relative to wild type as determined in liquid culture.

If **1A08** targets the DDCR pathway, the deletion strains that are sensitive to **1A08** should be enriched in genes that have SGIs with the DDCR pathway. In other words, while genes directly sensitive to **1A08** may not be enriched for any particular pathway, they should exhibit significant SGIs with the DDCR pathway. We took the 100 most sensitive deletion strains to **1A08** as detected in the genome-wide sensitivity screen, and found 95 other deletion strains that shared a significant number of SGI connections to the original 100 genes based on a hypergeometric test (see Supplemental Information). We next asked whether the 95 “SGI neighbors” of the **1A08**-sensitive genes (that is, the genes that share SGIs with **1A08**-sensitive genes) were overrepresented by any pathway. We overlapped 1273 GO categories with these 95 genes and included the DDCR pathway constructed from several GO categories as previously described. 153 GO categories had p-values less than 0.05, of which 21 categories had p-values less than the Bonferroni-corrected 0.05 level ($P < 10^{-4.4}$). As expected, the DDCR pathway had the most significant overlap to the SGI neighbors out of the 4347 Gene Ontology categories tested (Figure 9, Table 2). Interestingly, in contrast to their SGI neighbors, the genes directly sensitive to **1A08** are more likely to belong to non-specific multi-drug resistant pathways such as membrane transport and vacuole-related pathways. Out of the 40 genes that have known SGIs with *SOD1*, 27 are also SGI neighbors of the **1A08**-sensitive deletions. Thus, the sensitivity profile of **1A08** closely matches the computational prediction using the compendium of SGIs.

Activity of **1A08** in human HCT-116 cells

Since many checkpoints and repair processes are highly conserved between yeast and humans, we tested the effect of **1A08** on human cells alone and in combination with the DNA damaging drug camptothecin. The HCT-116 line was chosen since these cells have active DNA-damage checkpoints[30,31], and CPT was used as a DNA damage-inducing agent since it has been shown to trigger the G1, intra-S-phase, and G2/M checkpoints in a dose dependence manner[32]. The CPT-induced DNA damage response was confirmed in HCT-116 cells by incubating with different concentrations of CPT for 24 h and analyzing the cell cycle distribution by flow cytometry. As expected, the cell cycle distribution varied with CPT concentration, from an increased cell count in G2/M (10 nM), to intra-S-phase (50 nM), and finally to a buildup of cells in G1 (>200 nM) (data not shown).

1A08 on its own was cytotoxic to HCT-116 cells over 24 h, with an LD₅₀ of 7.5 μ M (Figure 10). We next observed that **1A08** was able to abrogate the CPT-induced DNA damage checkpoint in S-phase. To HCT-116 (p53 +/+) cells, 50 nM CPT was added in combination with DMSO, **1A08**, or caffeine (a known checkpoint inhibitor) for 24 h. A significant shift

in cell cycle distribution was observed from S-phase to G2/M in the **1A08**- and caffeine-treated samples. This response was dose-dependent, with the percentage of cells in S-phase decreasing with increasing concentrations of **1A08**, from 47.8 $\pm$ 3.3% in the presence of CPT alone to 23.0 $\pm$ 1.9% with the addition of 10 μ M **1A08** (Figure 11A).

To verify that the observed change in cell cycle distribution was due to a disruption of the S-phase DNA damage checkpoint, a BrdU incorporation assay was used to measure new DNA synthesis in the presence of CPT and **1A08**. We grew HCT-116 (p53 $+/+$) cells in a 96-well plate, and treated them with 50 nM CPT and 10 μ M BrdU in combination with either caffeine or various concentrations of **1A08**. In the presence of increasing concentrations of **1A08**, BrdU incorporation increased with a maximal effect near its LD₅₀ of 7.5 μ M (Figure 11B).

We also tested whether **1A08** abrogates the G1 DNA damage checkpoints by incubating HCT-116 (p53 $+/+$) cells with 200 nM CPT for 6 h in the presence or absence of **1A08**. Nocodazole was included to prevent passage through mitosis, and caffeine was used as a positive control to verify that checkpoint disruption was observable by flow cytometry. We saw no effect by **1A08** on the amount of cells in G1 induced by 200 nM CPT (Figure 11C). A dose of 2 mM caffeine, on the other hand, abrogates the CPT-induced checkpoint in G1 significantly, leading to an increase in the relative population of cells in S-phase and G2/M (Figure 11C).

Finally, **1A08** was tested for its ability to abrogate the G2 DNA damage checkpoint. The G2 checkpoint is activated separately by p53-dependent and p53-independent mechanisms[33]. While caffeine, which inhibits ATM/ATR checkpoint kinases, is capable of overriding the G2 DNA damage checkpoint in p53 $-/-$ cells, p53 $+/+$ cells are relatively insensitive to the effects of caffeine[33]. To test the effect of **1A08** on the G2 checkpoint, HCT-116 p53 $+/+$ and $-/-$ cells were treated for 24 h with 10 nM CPT, along with either caffeine or 5 μ M **1A08**. Nocodazole was added to prevent cells from passing through mitosis, and the ratio of mitotic to interphase cells were counted by microscopy using a fluorescent DNA stain. In the p53 $+/+$ cells, we saw that neither **1A08** nor caffeine abrogated the G2/M checkpoint induced by 10 nM CPT (data not shown). For the p53 $-/-$ cells, caffeine did abrogate the G2/M checkpoint (consistent with reported observations) while **1A08** had no effect (Figure 11D). Taken together, this data strongly supports the hypothesis that **1A08** is a specific inhibitor of the intra-S-phase DNA damage checkpoint in the human cell line HCT-116.

Discussion

High-throughput screening in yeast is relatively inexpensive and can be performed on multiple mutants in parallel with the parental wt strain. In principle, this method can be generalized to other pathways of interest, provided that a large degree of sequence and/or structural homology exists between the yeast and human targets. As our screening strain, we selected *sod1* yeast, which had known SGIs with 27 of the 210 genes listed in the DDCR pathway. Since the selection criteria were based on the compendium of known SGIs in the public domain, the approach is limited somewhat by the quality and completeness of the data. Until the database of SGIs reaches saturation and every yeast double mutant has been

scored for fitness, the selection of the most informative screening strain for a given pathway will inevitably be associated with some uncertainty. In principle, greater coverage of a pathway would be obtained by expanding the number of deletions screened. Other deletion strains could be included as negative controls, which, along with wt, could be used to exclude off-pathway hits in the initial screening phase. In this case, we identified many oxidizing or known ROS-producing compounds that were later filtered out in secondary screens for anti-DDCR activity in yeast and human cells.

1A08 is the Diels-Alder product of tetracyanoethylene and cyclohepta[*cd*]benzofuran[34]. No biological activity is associated with **1A08** in the literature. However, an online analysis using the National Cancer Institute's COMPARE analysis tools showed good correlations between **1A08** and two Hsp90 inhibitors: macbecin II, a geldanamycin derivative (correlation = 0.78), and rifamycin (correlation = 0.68). The COMPARE analysis correlates toxicity profiles in the NCI Human Tumor Cell Line Screen, and these correlations between compounds can indicate similar mechanisms of action[35]. The correlation between **1A08** and Hsp90 inhibitors may reflect the role of Hsp90 in protecting the cell from DNA damage by modulating the stability of a number of DDCR-related proteins[36].

This proof-of-concept study has shown the value of combining bioinformatics and chemical screening to target the DDCR pathway in yeast, which, in this case, translated to the ability to abrogate DDCR signaling in human cells. Clearly, identification of the molecular target of **1A08** would greatly increase its utility as a molecular probe. While the DDCR pathway was significantly enriched among **1A08**'s SGI neighbors, no single gene emerged as a candidate target. As the completeness of the SGA grid increases, we expect target identification to be feasible by overlaying the homozygous genome-wide sensitivity data for **1A08** against the grid of SGIs. For some compounds, this approach may be limited by the possibility that inhibition by a small molecule may exhibit a different genome-wide signature when compared with the analogous genetic deletions. A combination of this approach and high-throughput target identification methods[37–40] would allow the paring down of candidate targets to a smaller number that can be tested in biochemical and/or functional assays. Despite these caveats, the expanding set of genome-wide genetic and chemical-genetic interactions ensures that yeast will continue to provide a powerful tool for small molecule discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Identification of “pathway hubs” p-hubs based on the synthetic genetic network
 Two genes, G_1 and G_2 , are considered as candidate p-hubs to the target pathway P_1 . Genes (circles) are scored according to how specific their synthetic genetic interactions (solid lines) are to genes from a target pathway, P_1 (see Methods). G_1 has a higher p-hub score (10) than G_2 (1.9) since G_1 has interactions more specifically associated with P_1 . The hypothesis of this work is that a drug that is selectively lethal to a deletion strain G_1 (relative to wt) is more likely to target P_1 than a drug that is selective for G_2 , since such a drug may target other pathways such as pathway P_2 .

Figure 2. Pathway Genetic Interaction (PGI) correlates with differential sensitivity (DSS)

For each of 15 positive control pathways, genes were grouped into three categories based on their PGI scores to the pathway – disconnected (D; PGI = 0), moderately connected (M; 0 PGI < 3), or highly connected (H; PGI > 3). Both positive (top twelve) and negative (bottom three) pathways were included in the analysis. Boxplots represent the distribution of DSS for genes in each group: disconnected (top boxplot marked D), moderately connected (middle boxplot marked M), and highly connected (bottom boxplot marked H). Boxplots represent the distribution of differential sensitivity, where each point represents how sensitive a gene's mutant is to compounds known to target the pathway compared to those that do not; line is the median level; edges represent the upper- and lower-quartiles; points show extreme values. Vertical column annotates whether genes with medium to high PGI had higher DSS on average than genes with lower PGI. Large upper quartile for genes highly connected to membrane biogenesis not shown in the plot.

Figure 3. Global overview of indicator genes for several pathways

Purple gradient shows increasing PGI score representing the significance of synthetic-lethal overlap of a gene (row) with a target pathway (column). Genes (rows) were clustered according to their PGI scores, and pathways (columns) were clustered according to PGI scores across all genes (left matrix) or binary membership of those genes (right matrix). All genes predicted as an indicator for at least one pathway are included. Inset shows detail of cluster F, a set of genes with specific synthetic lethal interactions to genes of the DNA damage and DNA replication pathways. SOD1 has the highest PGI score for the DNA damage-related pathways and is not a member of one of these pathways. All of the pathways associated with each pathway cluster i through xv are available as Table S3; all of the genes in clusters A through S are available as Table S4.

Figure 4. Identification of screening candidates for the damage pathway

A. Genome-wide scores for screening candidates. Each gene was scored according to how significant its synthetic genetic interaction (SGI) partners overlapped with genes in the damage pathway. Plotted is the number of damage pathway genes that also are linked to the gene against the significance of this overlap computed as $-\log_{10}(P)$, where P is the P -value given by the hypergeometric distribution (see Methods).

B. *SOD1*'s synthetic genetic defect neighborhood. The SGI neighborhood of the gene, *SOD1* (circles linked to *SOD1*), was found to have the most significant overlap with genes in the damage pathway (orange circles). Lines depict SGI links; blue circles represent genes that are synthetically defective with *SOD1* but are not known to belong to the damage pathway.

Figure 5. Structures of compounds that selectively kill *sod1⁻* yeast
NSC numbers are given for the 15 compounds.

Figure 6. 1A08 Disrupts Normal DNA Damage Responses in Wild Type Yeast

A. Spot assays with **1A08**. 0.5 μ l of a 40 mM solution of **1A08** were spotted onto filter discs on top of soft agar containing yeast and the following: i) Untreated plate; ii) 0.01% MMS; iii) 0.02% MMS; iv) 250 μ M cisplatin; v) 1 mM cisplatin. B. Liquid culture validation of spot assays. The effect on growth in liquid culture was measured for **1A08** at 10 μ M and 25 μ M in the presence of 0.02% MMS.

Figure 7.

The sensitivities of 4759 homozygous deletion mutants to 17 μ M **1A08** is shown. The experiments were performed as reported[15]. Sensitivities, relative to the DMSO control, are plotted on the y-axis for each homozygous deletion strain (arranged alphabetically according to gene name on the x-axis).

Figure 8. Growth of wild type and *sod1* yeast in the presence of 1A08
A. Wild-type. **B.** *sod1* .

Figure 9. SGI neighbors of 1A08-sensitive genes enriched for DNA damage checkpoint and repair

Each Gene Ontology (GO) category was overlapped with both **1A08**-sensitive deletions (x-axis; $-\log$ P-value of overlap), and significant SGI “neighbors” of **1A08**-sensitive deletions (y-axis; $-\log$ P-value of overlap). Only GO categories with a Bonferroni-corrected P value < 0.05 for either hits or neighbors are shown.

Figure 10. Growth Inhibition of 1A08 on Log Phase Growing HCT-116 Cells

Cells were treated with various concentrations of **1A08**, from 250 nM to 20 μM, for 24 h. MTT was added for 3 h before washing cells and dissolving them in DMSO. Absorbance at 570 nm was measured using a plate reader.

Figure 11. 1A08 is a Specific Inhibitor of the Intra-S-Phase DNA Damage Checkpoint

A. HCT-116 cells show a dose dependent response for escape from S-phase in the presence of 50 nM CPT after 24 h. **1A08** was added at the indicated concentrations. Caffeine was added at 2 mM. **B.** Incorporation of Brd-U in the presence of 50 nM CPT is also dependent upon 1A08 concentration, with the strongest activity near the LD50 value of untreated cells. **C.** The percentage of cells in G1 does not significantly change when treated with 5 μ M 1A08 for 6 h in the presence of 200 nM CPT, black bars show G1, white bars show S-phase, and grey bars show G2/M. **D.** The percentage of mitotic cells in HCT-116 p53 $-/-$ cells does not change significantly when treated with 5 μ M 1A08 in the presence of 10 nM CPT, black bars show interphase cells, grey bars show mitotic cells. Error bars represent standard deviations from the average of three independent experimental replicates.

Table 1

Twenty-seven control compounds with known target pathways.

Target Pathway	Compound(s) Assayed in Hillenmeyer	Control Type ^a
actin	wiskostatin, latrunculin	+
sphingolipid biosynthesis	aureobasidin A, myriocin	+
Anti-metabolite	methotrexate, aminopterin	+
translation elongation	cycloheximide	+
membrane biogenesis	miconazole, clotrimazole	+
cell wall	amphotericin B, caspofungin, nystatin	+
peptidyl-prolyl cis-trans isomerase activity	fk506	+
TOR signaling	rapamycin	+
microtubules	benomyl, nocodazole, rhizoxin	+
oxidation	berberine chloride, xanthohumol	+
proteasome	aclacinomycin A	+
histone deacetylase	Splitomicin	+
phosphatase inhibitor	calyculin A	– ; non-specific target set
DNA damaging	acriflavinium hydrochloride, cisplatin, oxaliplatin, carboplatin, chlorambucil	– ; targets DNA, not gene product

^aControl Type. Both Positive (+) and negative (–) controls that were also profiled in the Hillenmeyer *et al.* (2008) dataset [6] were selected. Positive controls target a specific gene product while negatives have a diverse range of targets.

Table 2

The top p-hubs for several pathways.

Target Pathway	ORF	Symbol	Description	Score ^a	OV ^b	PI ^c	GI ^d
chromosome organization and biogenesis	YJL115W	ASF1	Nucleosome assembly factor	37.2	80	571	140
DNA packaging	YOL012C	HTZ1	Histone variant H2AZ, exchanged for histone H2A in nucleosomes by the SWR1 complex	31.2	48	252	114
vesicle-mediated transport	YNL267W	PIK1	Phosphatidylinositol 4-kinase	29.9	29	333	32
DNA damage checkpoint & repair	YJR104C	SOD1	Cytosolic superoxide dismutase	27.7	27	198	40
mitotic cell cycle	YER016W	BIM1	Microtubule-binding protein	26.6	59	256	212
DNA replication	YDR217C	RAD9	DNA damage-dependent checkpoint protein	24.4	23	129	44
M phase	YPR135W	CTF4	Chromatin-associated protein	23.8	54	265	191
chromosome segregation	YDR014W	RAD61	Sister chromatid cohesion	22.7	16	121	19
			5' to 3' exonuclease, 5' flap endonuclease, required for Okazaki fragment processing and maturation				
DNA recombination	YKL113C	RAD27		20.9	35	120	187
establishment of nucleus localization	YOR349W	CIN1	Tubulin folding factor D	19.5	12	20	47
cytoskeleton organization and biogenesis	YEL003W	GIM4	Subunit of the heterohexameric co-chaperone prefoldin complex	19.4	39	236	134
protein amino acid alkylation	YKL139W	CTK1	Catalytic (alpha) subunit of C-terminal domain kinase I (CTDK-I)	19.4	13	23	57
nuclear transport	YLR335W	NUP2	Nucleoporin involved in nucleocytoplasmic transport	17.7	12	130	13
membrane fusion	YNR049C	MSO1	Probable component of the secretory vesicle docking complex	17.5	10	58	12
RNA splicing	YKL173W	SNU114	GTPase component of U5 snRNP	17.3	11	127	11

^aScore. The Pathway Genetic Interaction (PGI) score.

^bOV. The number of overlapping synthetic lethal interactions between the gene and the pathway.

^cPI. The number of total synthetic lethal interactions calculated for the pathway.

^dGI. The number of total synthetic lethal interactions associated with the gene.