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Drug repurposing: a better approach for infectious disease drug discovery?

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The advent of publicly available databases containing system-wide phenotypic data of the host response to both drugs and pathogens, in conjunction with bioinformatics and computational methods now allows for *in silico* predictions of FDA-approved drugs as treatments against infection diseases. This systems biology approach captures the complexity of both the pathogen and drug host response in the form of expression patterns or molecular interaction networks without having to understand the underlying mechanisms of action. These drug repurposing techniques have been successful in identifying new drug candidates for several types of cancers and were recently used to identify potential therapeutics against influenza, the newly discovered Middle Eastern Respiratory Syndrome coronavirus and several parasitic diseases. These new approaches have the potential to significantly reduce both the time and cost for infectious diseases drug discovery.

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Introduction

Drug development research for infectious diseases has led to a number of effective therapies in the 20th century; however, there are still many diseases for which no drugs or vaccines are available. For other diseases, such as those caused by influenza virus or hepatitis C virus, treatments are suboptimal and effective for only a subset of the population. Reductionist or structure-based drug design efforts rely on *de novo* predictions of how a select set of small molecules or compounds will interact with targeted pathogen or host proteins. Such predictions are typically difficult, time consuming, and costly. Additional approaches are needed to discover new treatments and

to improve on existing ones. One promising approach is drug repurposing or repositioning; that is, applying known drugs or compounds to new indications [1,2]. Although this idea is not new, past techniques have relied on hypothesis-driven approaches that usually involve computational matching of compounds to specific viral or human proteins, requiring a large amount of expert knowledge on the chemical compound and drug target under study.

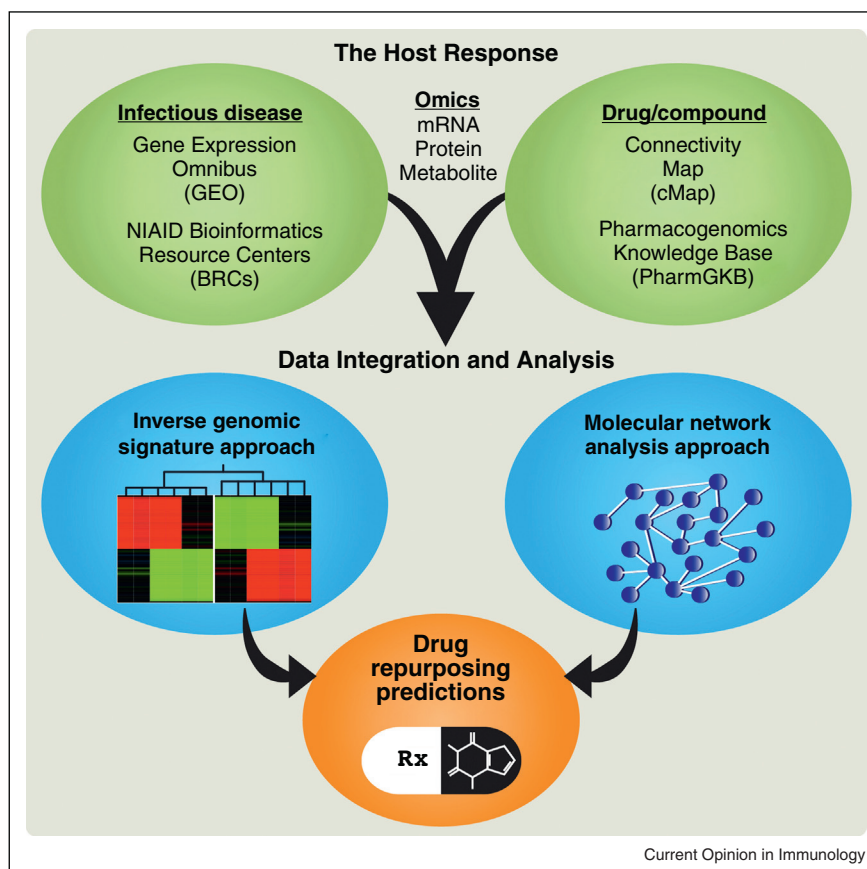
Recent developments have opened the door to using drug repurposing approaches that do not rely on generating empirical data related to binding characteristics or mechanism of action. Instead, these approaches use the methods of systems biology and bioinformatics to directly compare the host response to pathogen and drug. The computational methods used in this paradigm vary in complexity from genomic signature comparisons to complicated interaction networks. In combination with systematic databases of drug-induced gene expression profiles, these methods utilize data from a variety of high-throughput techniques (e.g., transcriptomics, proteomics, or metabolomics), thus allowing for the identification of potential host drug targets on a global-scale (Figure 1). Because of the significantly reduced time-frame for predicting host molecules for effective therapeutic intervention, and because these compounds are typically previously FDA-approved drugs or small molecules, these approaches have the potential to greatly reduce both the time and cost associated with drug development. Importantly, there has already been successful application of these approaches for several disease indications [3].

The time is therefore at hand for the infectious disease field to embrace a new paradigm in an effort to improve effective drug discovery. To illustrate the immediate accessibility and potential of this approach, we discuss examples both in and outside of the infectious disease field that have relied on systems-wide host response datasets, publicly available datasets of known drugs or small molecules, and computational approaches that are used to predict potentially effective disease–drug combinations.

Inverse genomic signature approach

In the simplest of terms, the inverse genomic signature approach is based on the premise that an effective drug generates a gene expression profile that is inversely

Figure 1



Components of the drug repurposing paradigm. System-wide phenotypic datasets, such as mRNA expression, proteomics and metabolomics, are collected for characterization of the host response to both drugs and pathogens and submitted to public repositories. This allows for data integration and comparisons using a variety of bioinformatics and computational methods, including inverse genomic signature and network-based approaches. These analyses result in drug repurposing predictions that identify potentially effective FDA-approved drugs as therapeutics for corresponding infection diseases.

correlated to the host signature associated with the disease. This approach incorporates the complexity of the genome-wide response of the host to both the disease and the treatment and is rooted in scalar theory [4^{*},5,6]. That is, the mRNA expression profiles contain information associated with higher-level protein interactions concordant with either the disease or drug treatment. Most examples of this approach use Connectivity Map (cMap) [7], a public database (<http://www.broadinstitute.org/cmap/>; version 2) that holds over 6000 transcriptome profiles established downstream of treatment of human cell lines with over 1300 compounds, most of which are FDA-approved drugs. In addition to the transcriptome profiles, the cMap resource provides analytical tools, which among other things can calculate a 'connectivity score.' This score is a measure of the inverse similarity or negative correlation between signatures.

As an example, Dudley *et al.* used this method to identify new therapeutic agents against inflammatory bowel

disease (IBD), a progressive inflammatory disorder for which there is no known cure. Current treatments have severe side effects, are expensive, and are ineffective for many individuals [8]. In this study, a modified version of the computational methodology developed by Lamb *et al.* [7,9] was used to compare a compendium of 164 known drug compounds in cMap to an IBD-specific gene expression signature derived from 176 datasets available in Gene Expression Omnibus (GEO). Two of the strongest anti-correlated treatment signatures were from prednisolone, a well-known treatment for Crohn's disease (a major type of IBD), and topiramate, an anticonvulsant drug used to treat epilepsy. Using a rodent model of IBD, it was shown that topiramate significantly reduced gross pathology and microscopic damage in the affected colon compared with that seen in control animals. Importantly, topiramate's side effects in treatment of neurological symptoms in humans are not as severe as current IBD treatments.

In one of the first applications of this approach to an infectious disease, Josset *et al.* used the host transcriptional response to influenza virus to query the cMap database [10]. The majority of current antiviral drugs target specific viral proteins, making them narrow in spectrum and vulnerable to the emergence of viral resistance. Josset and co-workers reasoned that treating virus infection by stimulating or manipulating the innate immune response, or by targeting cellular factors required for the viral life cycle, would prove advantageous over the more traditional approach of targeting a viral protein. A common gene expression signature consisting of 20 dysregulated genes was identified from human A549 lung epithelial cells infected with human (H1N1 or H3N2) or avian (H5N1, H5N2, or H7N1) influenza A virus. The cMap database was then queried to identify candidate compounds with genomic signatures that were inversely correlated to the common influenza virus-induced host transcriptome profile. Eight candidate drugs were identified, the majority of which are approved for other indications. Six of these drugs inhibited viral growth in *in vitro* assays. Importantly, five out of the eight also inhibited the growth of the pandemic 2009 H1N1 influenza virus, which had not been used to define the common signature. The approach therefore has the potential to identify drugs effective against a spectrum of related viruses.

An additional exciting aspect of the inverse genomic signature approach is its ability to rapidly identify potential drugs for emerging infectious diseases, as demonstrated by a recent study from our laboratory in which we determined the host response to the newly discovered Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) [11^{*}]. MERS-CoV was first identified in Saudi Arabia in September 2012 and has since caused at least 77 confirmed infections resulting in 40 deaths. Using human Calu-3 cells (a cell line derived from epithelial cells lining the human conducting airway) and global transcriptional profiling of the host response, a 207-gene signature of the early host response was identified. Using this signature and two independent bioinformatics tools, the cMap connectivity score and IPA upstream regulator analysis (Ingenuity Systems, www.ingenuity.com), two kinase inhibitors (SB203580 and LY294002) were identified as potential anti-MERS-CoV treatments. To validate the top predicted negative regulator, cells were treated with SB203580 before MERS-CoV infection. This resulted in a significant reduction in viral titers at both 24 and 48 hours post infection. This study demonstrates the potential of *in silico* approaches to predict drug candidates and the *in vitro* efficacy of SB203580 against MERS-CoV.

Network-based approaches

Whereas the studies just described focused on the use of gene expression patterns, it is also possible to take advantage of the deeper understanding of the host

response that comes from network modeling. Biomolecular networks connect molecular components, such as genes, proteins, or metabolites by specific types of interactions and are used to represent the functional relationship among the various components of the network [12]. Functional relationships can be physical or chemical interactions, genetic regulatory interactions, or other associations. Networks may be constructed to capture various scales, ranging from molecular and cellular levels to tissue and organismal levels, and network dynamics can be analyzed to provide information about complex diseases, infer novel relationships, and reveal emergent properties of the biological system.

The application of systems biology to infectious disease research is increasingly being used to identify host targets for antimicrobial therapeutics and for the prediction of novel pathogen virulence factors [13–15]. With the wealth of functional genomics data that are now publicly available, network pharmacology can begin to make inroads into infectious disease research. Although an in-depth review of network-based approaches for drug repurposing is beyond the scope of this article, several recent reviews have focused on the complexity of human disease networks [16], tools for analyzing network topology and dynamics [4^{*}], and network and drug combinations [17]. Here, we discuss recent studies that have used network-based approaches for target identification and drug discovery.

To identify novel genes for drug targeting, Barrenas *et al.* [18] built upon the knowledge that genes containing disease-associated SNPs tend to form highly connected clusters in protein–protein interaction networks, as do genes that are differentially expressed in complex diseases. This information was used to identify highly interconnected gene clusters or ‘core-susceptibility modules’ from 13 highly diverse complex diseases. This was done by constructing individual disease-specific networks using a global human protein–protein interaction network and gene expression datasets for each disease [19]. Highly interconnected clusters that overlapped between the disease networks were then determined, and the use of a permutation test revealed a significant level of SNP enrichment (obtained from [20]) in each core-susceptibility module compared with the whole protein–protein interaction network.

To test if this correlation between core-susceptibility modules and SNPs could identify novel host drug targets or novel genes associated with disease susceptibility, the network construction and analysis was repeated using gene expression and SNP datasets from another complex disease, seasonal allergic rhinitis (SAR, i.e., hay fever). This analysis identified two novel SAR-associated genes, fibroblast growth factor 2 (*FGF2*) and mitogen-activated protein kinase 8 (*MAPK8*), the latter known to play a role

in type 1 allergic inflammation. The potential functional relevance of *FGF2* as a drug target was confirmed when it was knocked down in polarized TH2 cells, which resulted in the dysregulation of genes involved in type 1 allergic inflammation.

Drug repurposing strategies may also be valuable for finding therapeutics against rare diseases, where there is little financial incentive for drug development. As an example, Mei *et al.* [21] used a multiple-level network modeling (MLNM) approach to identify drugs to treat rare central nervous systems (CNS) diseases. Using publicly available disease-gene expression profiles, pathophysiological pathway analysis, and information on FDA-approved drugs and late-stage compounds, an integrated CNS disease network was constructed in which diseases were linked in relationship to the degree of commonly shared pathways, expression patterns, or responses to drugs or compounds. Using robust association statistical cutoffs, hierarchical clustering, and the hypothesis that similar diseases are caused by the dysregulation of related pathways, several potential drugs were identified. For example, these results predicted that Parkinson's disease drugs could be effectively repurposed as a therapy for basal ganglia disease. Similarly, this approach showed that multiple sclerosis (MS) and neuromyelitis optica (an autoimmune disorder consisting of simultaneous inflammation and demyelination of the optic nerve and the spinal cord) share common mechanistic pathways, suggesting that MS drugs could be effective against both diseases.

In a novel approach, Daminelli *et al.* used off-target effects of known promiscuous drugs to their advantage in a network-based strategy that integrated publicly available drug–target and drug–disease interactions [22]. Using the resulting integrated drug–target–disease network, new links between drugs, targets, and diseases were identified and evaluated. For example, quercetin, a bioflavonoid with anti-inflammatory and antioxidant properties [23], and resveratrol (a polyphenolic compound found in red wine and once speculated to explain the 'French Paradox' [24]) were predicted to bind the same two targets (PIK3GC and LTA4H, known binding partners of quercetin and resveratrol, respectively) and potentially be effective against neoplasms and cardiovascular diseases. A literature search revealed that quercetin binding to LTA4H had recently been verified using an *in vitro* pull-down assay. Although resveratrol does not appear to bind directly to PIK3GC, it can inhibit PIK3GC phosphorylation and has been reported to have anti-tumor activity [25–27].

Conclusions

Drug repurposing approaches therefore show promise for identifying drugs to treat a variety of diseases, including infectious disease. However, there are also caveats to be

aware of and steps to be taken to ensure that these approaches bear fruit. For instance, most if not all publicly available drug profiles have been obtained using cell lines as model systems, meaning that information related to intercellular mechanisms is not captured. Additional public funding is needed for more extensive databases that expand the number of drugs profiled and the model systems used. Equally important is the availability of datasets of the host response to infectious diseases. To this end, the National Institute of Allergy and Infectious Diseases (NIAID) has sponsored five systems biology centers for infectious disease research [28]. These centers focus on modeling the host response to pathogens such as influenza virus, SARS-CoV, *Mycobacterium tuberculosis*, *Salmonella typhimurium*, *Yersinia pestis*, and most recently malaria parasites. The resulting host-response datasets, along with a significant amount of metadata, are captured by the NIAID Bioinformatics Resource Centers, thereby allowing investigators to use these extensive, systematically obtained genomic, proteomic, and metabolomic datasets for computational studies.

There are also currently limitations to the computational tools used to integrate drug and host-response profiles. Existing pattern-matching techniques use prior knowledge in the form of gene ontology and functional annotation databases such as GO/KEGG or Ingenuity Pathway Analysis. This limits the searchable patterns to already known pathways. Additionally, cMap can only be searched with simple, short, differentially expressed gene lists rather than complex datasets obtained using multiple biological conditions and covering all human genes. This results in greater bias and lower throughput. Additional methods need to be developed to overcome these limitations. Of course, once potential drugs are identified through these approaches, steps must still be taken to validate clinical efficacy [29].

In this review, we have highlighted the use of mRNA and proteomic profiles; however, other system-wide metrics could potentially be used, including profiles of microRNAs, long noncoding RNAs, or epigenetic modifications. With the recent US Supreme Court decision to ban patents on human genes, it is not hard to imagine that soon an individual's genetic makeup and personal genomic disease signatures will be screened against drug databases for a more tailored and personalized medical treatment. Finally, by identifying compounds that have already been evaluated in human subjects, drug repurposing approaches may be of particular benefit for identifying treatments that can be employed against rare or emerging infectious diseases. As an adjunct to the more common approaches of high-throughput screening and structure-based drug design, computational drug repurposing has the potential to rapidly translate insights gained through systems biology into a drug discovery

strategy directly applicable to the treatment of infectious disease.

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