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Systems Pharmacology

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Abstract

We examine how physiology and pathophysiology are studied from a systems perspective, using high-throughput experiments and computational analysis of regulatory networks. We describe the integration of these analyses with pharmacology, which leads to new understanding of drug action and enables drug discovery for complex diseases. Network studies of drug-target relationships can serve as an indication on the general trends in the approved drugs and the drug-discovery progress. There is a growing number of targeted therapies approved and in the pipeline, which meets a new set of problems with efficacy and adverse effects. The pitfalls of these mechanistically based drugs are described, along with how a systems view of drug action is increasingly important to uncover intricate signaling mechanisms that play an important part in drug action, resistance mechanisms, and off-target effects. Computational methodologies enable the classification of drugs according to their structures and to which proteins they bind. Recent studies have combined the structural analyses with analysis of regulatory networks to make predictions about the therapeutic effects of drugs for complex diseases and possible off-target effects.

Keywords

drugome; signaling networks; systems biology; systems pharmacology; targeted therapy

Classical studies such as the development of receptor theory by Clark¹ and Black,^{2,3} followed by analyses that distinguished between competitive and noncompetitive inhibition, served as the initial insight on the molecular mechanisms of drug action.⁴ The influence and relevance of receptor theory in modern pharmacology are due to the large number of drugs that target membrane receptors, mostly G-protein-coupled receptors (GPCRs). A majority of the remaining drugs are enzyme inhibitors, which are generally substrate-based analogs, transition-state analogs, or allosteric inhibitors, designed based on the structures of the substrate and the substrate-binding pocket to bind reversibly or irreversibly. Over the past decade, genomics, proteomics, network analyses, and other high-throughput studies have led to a wealth of “systems-level” information. Through combinations of the human genome studies, high-throughput technologies, and structural and biochemical studies, the number of well-characterized, druggable targets is constantly increasing.^{5,6} Hence, the drug pipeline has grown, with a focus on complex, multigenic diseases resulting in the appearance of targeted therapies and biological therapeutics, such as kinase inhibitors and monoclonal antibody therapies. Despite these advances, the overall rate of development of new drugs has greatly decreased.

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DISCLOSURES

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There are more than 1500 drugs approved by the US Food and Drug Administration (FDA), but only approximately 400 unique proteins are targeted by these approved drugs (the “drugome”) (Figure 1). The diseaseome is defined as the current ~3700 unique entries in the Online Mendelian Inheritance in Man (OMIM) database and covers a tiny fraction of the proteome, which is estimated at approximately 100,000 proteins. The research drugome (the proteins targeted by approved drugs and/or drugs in research and clinical trials) overlaps with a portion (500 protein targets) of the diseaseome (Figure 1), and is focused in 15 to 20 therapeutic areas.^{7,8} This means that about two-thirds of current drugs do not target a gene product that has been associated with disease.

The drug-discovery process is hindered by the cost and time of the research and development efforts necessary to take a drug from clinical trial to market: \$1 billion and from 5 to 10 years, on average. Furthermore, many drugs fail in phase III trials due to lack of efficacy (no significant difference between the placebo and drug treatment or between drug and current treatment alternative). Considering the extremely high cost of new-drug development and the high drug attrition rate, it is evident that new approaches are required to improve and expedite the process. Since most current drug-discovery efforts are targeted toward complex diseases, understanding the pathophysiology at a systems level could aid in the design and development of drugs with higher success rates in clinical trials.

The broadly defined field of systems biology has generated a large amount of accumulated knowledge on the biological processes involved in disease and drug action. In this review, we will provide an overview of how systems biology has been used to analyze the molecular mechanisms of human disease, current drug-target relationships, and mechanisms of targeted therapies, which is cumulatively termed “systems pharmacology.”

ANALYZING MECHANISMS OF DISEASE: THROUGH REGULATORY NETWORKS

It is currently understood that single-gene disorders are rare and most genetic diseases are caused by the concerted action of many genetic abnormalities, along with other possible nongenetic influences such as environmental factors, hormone levels, age, weight, and diet. Consequently, the treatment of diseases with a single, “magic bullet” therapy has proven largely unsuccessful. The current understanding of pathophysiology is complicated by the large amount of information that has amassed over the years from biochemical and cell-biological studies, and a systems view is necessary to integrate all the factors that control the regulatory networks involved in orchestrating the physiological and pathophysiological responses.

In order to gain a systems-level, molecular perspective of disease, a network view of the relevant physiological function is essential.

Each physiological process has an underlying signaling network of chemicals, hormones, native ligands, protein receptors, ions, enzymes, transcription factors, DNA with associated epigenetic mechanisms, and RNA, both regulatory and protein encoding. Together these components control the level of various cellular components to modulate biochemical reactions, electrical signals, force generation, transcription, and protein translation. These cellular processes can occur with different kinetics, simultaneously and/or at different times, and at varying levels of magnitude. Therefore, each physiological function or phenotype has a very complex network of signals controlling it. To represent each physiological process in a parsable network, each protein, ion, or chemical becomes a “node.” and each interaction between 2 nodes (binding, chemical reaction, or other interaction) becomes an “edge” (Figure 2A, Table 1). Signaling networks can be represented as undirected graphs wherein

interactions are specified but without hierarchy, or as directed graphs where the effect of 1 node on another is specified by the direction of the arrow. More specifically, directed graphs can have sign specification, such as positive for activation, negative for inhibition, or neutral interactions for scaffolds, which are represented differently by arrows, symbols, or colors. The hypothetical network in Figure 2A is directional, with activating interactions represented as arrows, inhibitory interactions as plungers, and neutral interactions as lines. In biological systems, signaling networks are not unidirectional⁹; instead, they are highly clustered. Certain nodes, termed “hubs” (Figure 2B), are highly connected,¹⁰ thus allowing many more components to be connected to other subnetworks. On the other hand, a system can contain “islands” (Figure 2B), sets of nodes not connected to the overall network. Several types of motifs exist within regulatory networks, such as negative feedback loops, positive feedback loops, and feed-forward loops (Figure 2B).⁹ These characteristics, as well as the high clustering coefficient, are attributed to the scale-free (a connectivity distribution that follows a power law¹¹) and redundant properties of biological networks. These properties also allow for network perturbation without complete loss of function. Thus, a single imbalance in the regulatory network of a biological system, such as an increased protein level or biochemical reaction rate, does not necessarily lead to a disease state. Instead, within the regulatory network of a disease system, the signaling networks underlying the pathophysiology are likely to be persistently perturbed at more than 1 point (edge or node).

The genomic irregularities associated with disease have been studied, through genome-wide association and positional cloning studies, which have become more efficient with the sequencing of the human genome. More recently, numerous genomic, short hairpin/small interfering RNA (shRNA/siRNA), proteomic, and phosphoproteomic studies have focused on the gene-expression signatures and protein-signaling pathways associated with a particular disease (Table 2).^{12–17} The plethora of data on the genetic mutations and variations produced by studies such as these has been compiled into several databases, such as the OMIM database and the Catalogue of Somatic Mutations in Cancer (COSMIC) database. These global databases help to identify genes, proteins, and other cellular components related to the origin or progression of a specific disease. These components can be used to generate lists of “seed nodes” that enable us to computationally construct various types of networks by integrating knowledge about gene-disease associations and/or protein interactions reported in biomedical literature. Through information about the properties of disease genes as nodes in a network, previously unrelated relationships between disease pathways and cellular functions can be identified, which can serve as a first step in identifying drug targets.^{18–20}

An interactive, computable database, the Connectivity Map,²¹ finds correlations between gene-expression signatures and the sets of proteins involved with the action of a class of drugs or with a particular disease. The initial Connectivity Map project analyzed the genome-wide messenger RNA (mRNA) expression profiles of 1 cell type after treatment with 164 small molecules and perturbagens (a subset was tested in 3 other cell lines). Instead of using traditional hierarchical clustering for analyzing the genetic data, the authors developed and adopted a method termed “gene set enrichment analysis,” a nonparametric, rank-based pattern-matching strategy,²¹ with the idea that regardless of the cell type, the gene-expression data specific to perturbation by a small molecule could be detected. The gene-expression signatures could be queried to search for commonalities among expression signatures for seemingly unrelated drugs or drug classes. This tool aids in the empirical understanding of current drug action and the discovery and characterization of new drug entities.²²

In another example, Goh *et al.*¹⁹ examined gene-disorder association data in 2 networks: (1) the human-disease network, which treats human diseases as nodes, connected based on the associated genes they have in common, and (2) the disease-gene network, which connects disease genes according to whether they are associated with the same disorder. The data were used to construct a bipartite interaction network using 1284 known disorders and 1177 known disease genes from the OMIM database. The diseases were further broken down into 22 classes. Both networks were highly connected and had a “giant component,” a hub-like area where many nodes were connected to one another. In the human disease network, cancer and related disorders and neurological disorders were in highly connected, heterogeneous hubs, whereas metabolic, skeletal, and other disorders were in lesser-connected “islands.” With a wide distribution of gene associations, most diseases associate with a few genes, whereas some disorders associated with a much higher number of genes (30 to 50). There were numerous islands, more notably in the disease-gene network. It was previously demonstrated that disease-related genes are hubs or have a higher number of interactions than nondisease proteins.

The majority of the gene list used in the human disease and disease gene networks contained nonessential genes (“essential” defined as being embryonic/postnatal lethal).¹⁹ It was observed that with essential genes excluded from the disease-gene network, the connectivity greatly diminishes, indicating that the higher connectivity was observed solely due to the high connectivity inherent to the essential genes. Furthermore, the expression patterns of disease genes were asynchronous with genes essential for cellular functions, and the disease genes had little overlap with housekeeping genes. This indicated that disease genes are often peripheral, with 1 important exception: somatic mutations found in cancer, which did not fit the analysis described above.

In another analysis of the properties of disease genes from a systems perspective, Zhong *et al.*²⁰ showed that genetic mutations that perturb networks at an edge (edgetic: mutated nearly full-length or point-mutated full-length gene products) rather than at a node (null: truncated gene products) are more heavily responsible for the phenotypic manifestation of the diseases.²⁰ Edgetic perturbations are more commonly found in mutations that span multiple diseases and are caused by in-frame mutations and the expression of a nearly full-length gene product, which lacks an interaction with a neighboring node (Figure 2C). In contrast, node removal or a null mutation includes severely truncated gene products or other mutations that destabilize protein structure and equate to the removal of the protein with respect to its function and interactions (Figure 2C). Edgetic perturbations have potential consequences at more than 1 node in the network. Furthermore, the authors cited examples where different mutations of the same gene product affect different edges, and each edgetic mutation is associated with a different disorder.

ANALYZING DRUG-TARGET RELATIONSHIPS WITH REGULATORY NETWORKS

Systematically organizing relationships between diseases, drugs, and proteins into a map such as a signaling network enables statistical inferences about less-obvious relationships between them. Global analyses of FDA-approved drugs have found that, unlike ubiquitously expressed essential genes, drug targets tend to be expressed in specific tissues but interact with many other proteins in the cellular network while being independently regulated.⁸ Commonalities of signaling mechanisms among existing drug targets create a basis for identifying the network properties that define a potentially good drug target. Statistical metrics from network analyses, such as a centrality measure, which quantifies the relative importance of a protein in communicating between different modules within a network, have

been suggested for identifying nodes (proteins in a network) that have attractive properties as potential drug targets.²³

Ma'ayan *et al.* constructed a bipartite graph of 1052 FDA-approved drugs interacting with 485 targets contained 179 islands, using data from DrugBank.^{24,25} Most of these islands are made up of 10 to 30 interacting cellular components. Drugs that target GPCRs comprise a single large island of 481 components.²⁶ A majority of targets of currently approved drugs are GPCRs (~25%),⁵ which govern a diversity of physiological processes such as cardiac contractility, acid secretion, and airway constriction.

In a study by Yildirim *et al.*,⁸ a bipartite graph of drugs and their targets listed in DrugBank was generated. Analysis of this drug-target network showed that the majority (62%) of currently approved drugs target membrane proteins, most likely the bulk of which are GPCRs, as indicated by the results of study by Ma'ayan *et al.*²⁶ In the analysis of the distribution of the targets of research and current drugs, the drug-target identity distribution became more diverse, with cytoplasmic targets doubling (6% to 12%). This result is indicative that drug-discovery technologies have improved to target nonmembrane proteins and the emergence of targeted therapies.

TARGETED THERAPIES: ADVANTAGES AND PITFALLS

Targeted therapies or signal transduction inhibitors make up the bulk of the current drug pipeline. Of all drug targets, the majority (25%) target GPCRs, followed by kinases (10%).⁵ The shift from GPCR drugs to other receptor classes and protein kinase-targeted drugs (>100 kinase inhibitors are in development and 15 are currently approved as cancer therapies, Table 3) is representative of the cumulative knowledge of the mechanisms underlying disease. The obvious advantage of targeted therapies is their mechanistic derivation, targeted to the specific signaling molecule (typically a kinase) that exhibits aberrant behavior in disease. Many targeted therapies target a new area and the risk of failure is higher if the drug has a novel mechanism of action.²⁷ Other disadvantages lie in the difficulty of achieving high specificity and the resulting off-target effects and primary and acquired mechanisms of resistance to these therapies. Complex signaling mechanisms and motifs often play a role in resistance, underscoring the importance of a systems-pharmacology understanding in the target choice and drug design.

The bulk of targeted therapies is used to treat cancer, which this discussion will focus on. The first example of a targeted therapy in cancer was tamoxifen, which specifically inhibits the estrogen receptor in estrogen receptor-positive breast cancers.²⁸ The first successful kinase inhibitor was the BCR-ABL inhibitor imatinib, which is approved for treating chronic myelogenous leukemia. Other examples (Table 2) include human epidermal growth factor receptor family (EGFR and HER-2/ErbB2) inhibitors such as monoclonal antibody therapies trastuzumab, cetuximab, and panitumumab, and the tyrosine kinase inhibitors erlotinib, gefitinib, and lapatinib.

By design, these therapies are administered to select patient populations that exhibit a specific mutation or heightened expression of the protein target. For example, prior to receiving trastuzumab, patients must test positive for elevated ErbB2 expression^{29,30}; and to be enrolled in a cetuximab combination clinical trial for pancreatic cancer, biopsies must show heightened EGFR expression.³¹ On the other hand, patients are also tested for primary mechanisms of resistance to these drugs, such as K-Ras mutations mitigating the response to EGFR inhibitors. Because these drugs target a specific signaling molecule, acquired resistance is a significant problem. For example, the T790M mutation in EGFR increases the affinity of adenosine triphosphate (ATP) for the EGFR active site but ablates the affinity of the EGFR kinase inhibitor gefitinib. Also, various mutations in mitogen-activated/

extracellular signal-regulated kinase kinase (MEK) were exhibited by tumors which relapsed following treatment with the MEK inhibitor AZD6244, which is currently in clinical trials.
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Other types of acquired resistance are caused by compensatory mechanisms, which are distinct from those encountered with nonselective chemotherapies which result from increase in the levels of the drug transporters like the ATP-binding cassette protein family. Instead, the resistance mechanisms involve nodes within the signaling network of the targeted protein. One example of resistance to EGFR therapy is the maintenance of phosphoinositide 3-kinase (PI3K) signaling through a variety of mechanisms, including loss of phosphatase and tensin homolog (PTEN) (a phosphatase and negative regulator of V-akt murine thymoma viral oncogene homolog [AKT]), ErbB3, or methionine (Met) amplification, which allows for tumor cell survival despite EGFR inhibition.³³ Additionally, in gefitinib-resistant and cetuximab-resistant cells, the HER signaling switches from EGFR-containing heterodimers and homodimers to primarily HER2-Her3 signaling dimers, which more potently activates the PI3K/AKT pathway.^{32,34}

In targeting a specific component in a complex signaling pathway, there is potential to disrupt all of the intrinsic signaling mechanisms associated with that particular node, which can have counterintuitive results.

For example, due to prevalence of mutations upstream of extracellular signal-regulated kinase (ERK) in cancers, such as mutant Ras and B-Raf, several B-Raf and MEK inhibitors are currently approved or in development. Due to the complexity of the signaling pathways, targeted inhibition of these molecules in cancer cells can cause unwanted signal amplification or bypass the inhibition within the targeted network. For instance, overactive B-Raf will hyperactivate the MEK–mitogen-activated protein kinase (MEK-MAPK) pathway, similar to oncogenic Ras, and inhibition of MEK should normalize this aberrancy, whether it originates with B-Raf or Ras mutations (Figure 3). However, MEK inhibition caused an increase in PI3K/AKT activation, and, vice versa, PI3K/AKT inhibition caused an increase in MAPK activation (Figure 3B).^{35,36} These paradoxical results are caused by a negative feedback loop in receptor tyrosine kinase (RTK) signaling that is mediated by TSC2-P70S6K and controlled by AKT and ERK. Hence, when MEK/ERK or PI3K/AKT is inhibited, a negative feedback loop is partially shut down, causing signal amplification in the noninhibited arm of the pathway (Figure 3B).

In another example, treatment with the MEK inhibitor (AZD6244) reduced proliferation cells that harbor the activating B-Raf V600E mutation.³⁷ However, in cells harboring activating K-Ras mutations with wild-type B-Raf, AZD6244 treatment caused accumulation of phospho-MEK, partially due to ablation of a negative feedback loop between MEK and B-Raf, mediated by Sprouty2 (Figure 3B).³⁸ This negative feedback loop is disrupted by the B-Raf V600E mutation, due to reduced affinity between mutant B-Raf and Sprouty2. Hence, response to AZD6244 is observed in cells harboring the V600E mutation.

Two recent, pertinent studies describe how B-Raf inhibition drives Ras-mediated B-Raf binding to and activation of C-Raf, followed by MEK-ERK activation (Figure 3C).^{39,40} In some B-Raf/Ras wild type (WT) cell lines and B-Raf WT/Ras-mutant cell lines, Raf-inhibitor treatment caused an increase in tumor cell proliferation. B-Raf inhibitor(s)' binding of the B-Raf ATP pocket potentiated the formation of B-Raf/C-Raf heterodimers at the membrane, in a Ras-dependent manner. Increased heterodimer formation correlated with increased C-Raf activity and increased MEK activation. MEK activation did not occur in drugs that inhibit both B-Raf and C-Raf. ³⁹ Another study on AKT inhibitors produced

similar results. When AKT was specifically inhibited, a pool of hyperphosphorylated AKT occurred, due to the “priming” caused by inhibitor binding the ATP pocket of AKT.⁴¹

These scenarios exemplify that network-signaling motifs play a role in drug action, highlighting the important role that the intricacies of the signaling pathways play in governing the effects of these targeted therapies.

A challenge in applying systems-pharmacology methods in translational medicine is the identification of complex signaling mechanisms early in the drug-discovery process. A first and necessary step to address this challenge in the clinic is obtaining as much data as possible on pertinent mutations and genomic variations, especially with complex diseases in which variable and multiple genetic factors lead to a diseased state. While a set of parameters does not exist to classify the gene products or interactions that will play a critical role in disease or drug action, establishing a systems perspective can lead to the detection of unforeseen effects of modulating a single component in a biological signaling network. In the early stages of considering a drug target, the following steps can be taken to enable a systems perspective and all the possibility of uncovering unforeseen consequences of modulating the activity of the drug target: (1) Using the literature and databases, identify all components that interact with the drug target of interest and the signaling pathways/networks with which these components are involved. (2) Construct a directed graph of all of these components (Figure 2), including all direct interactions and ones that lead to physiologically significant events (ie, ones that lead to a major result such as transcription, cell survival/death, motility, or others). (3) Identify redundant pathways and signaling motifs such as feedback loops, which may cause signal amplification, compensation, or other effects in the specific system of interest. (4) Establish a cell-based model to test these scenarios and take into account the results when considering the drug target and combining or prescribing drugs in a given genetic background.

COMPUTATIONAL METHODOLOGIES IN DRUG DISCOVERY

Often, drugs bind to several targets that are seemingly unrelated except for the target-binding pattern of the drug. Recent work has generated a network connecting drugs on the basis of their structural similarity and similarity of side-effect profiles.⁴² This method has been proven effective at identifying groups of drugs that share common targets. When drugs with predicted shared targets did not have known targets in common, the predictions provided a framework for testing these drugs for binding against the predicted targets. Hence, this approach led to the identification of new targets for existing drugs, which serve as new therapeutic targets or explain the cause(s) of an adverse effect.

There have been recent developments in “chemo-centric” methodologies to systematically compare the structures of drugs with known targets, as a means of predicting unknown target binding. The idea of searching a database of structures for a similar structure has been around a long time in the field of chemistry. For decades, chemists have been using structure-based literature searches for reaction methods, mechanisms, chemical information, and various analyses.⁴³ New approaches allow databases of structural and pharmacological data to be parsed using chemical structure, provide information on the global properties of compound libraries with respect to their biological properties, and predict binding interactions.

A major feat in establishing this methodology was the assembly of a large database of chemical entities, of which ~275,000 are curated with structure-activity relationship data (target identity, IC_{50} , EC_{50} , K_i , and K_D values) from >2800 targets.⁴⁴ The human subset of targets (700) and the respective drugs they bind was used to create a map of the protein targets (nodes) and how they are connected in chemical space (1 molecule binding 2

proteins creates an edge). The promiscuity of each target was analyzed, with aminergic GPCRs, cytochrome P450, and protein kinases ranking the highest. In a similar analysis, it was found that compounds generally targeted proteins from the same gene families. The method was also used to predict pharmacology of compounds and analyze how the physical properties of drugs differ within each target family. Unfortunately, the database is not publicly available and is proprietary to Pfizer, where the study took place,⁴⁴ but it represents a cumulative search tool, a novel method of searching for new leads in the drug-discovery process and improving the physical properties of lead compounds.

Another approach, coined the Similarity Ensemble Approach (SEA), classifies ligands, with known receptor interactions, by their chemical similarity using the Tanimoto coefficient of chemical similarity (Tc), a fragment-based algorithm.⁴⁵ Unrelated structures are parsed with these codes to predict with which protein targets they might interact. Comparing drug-target interactions by way of protein structure comparisons is much more difficult than by way of drug structure comparisons, due to the sheer difference in size and structural complexity. Central to this methodology is the idea that small molecules with similar structures will display similar target-binding properties. A recent study used this method and predicted that Paxil and Prozac (serotonin reuptake inhibitors) are also β -blockers.²³ The binding of these drugs to the β -adrenergic receptor was experimentally verified. The authors speculated that some observed adverse effects of the drugs, such as increased heart rate and sexual dysfunction, could be attributed to the off-target binding.

CONCLUSION

Basic cell biological research continues to delineate the functions of each protein in the proteome as well as metabolites in the metabolome to construct the “interactome” (how all cellular components interact) in both physiological and pathophysiological systems. The construction of the interactomes for specific pathophysiology can be quite useful for uncovering potential new drug targets. The combination of such physiological analyses at a systems level with informatics approaches based on drug structure and structure-function relationships offers a powerful new approach for drug discovery in the preclinical phase. The drug-discovery platforms have shifted toward targeted therapies, and drug-design technologies have greatly advanced to include biological therapies (antibodies and peptides), antisense therapies, and other methods to modulate protein-protein interactions, expression levels, or other interactions. However, more potential drug targets and drug-discovery technologies also means more possible efficacy failure and adverse effects that will set back the efforts to move drugs to the clinics. To enhance the rate at which new drugs are brought into the clinic, one will need to understand pharmacokinetic and pharmacodynamics of new drugs and use this knowledge in clinical-trial design. A systems-biology perspective and advanced computation methodologies provide an integrated basis for the understanding of the complex mechanisms of disease, targeted therapy action, and design combination therapies, and thus influence clinical-trial design to rapidly test new drugs in the clinic.

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Fig 1.

Relationships between proteins/genes involved with disease, drugs, and drug targets. The Venn diagrams represent the relationships between the drugome (the protein targets for currently approved drugs), the research drugome (the protein targets for drugs under research or in clinical trials), the diseaseome (the genes that have been associated with disease according to the Online Inheritance in Man [OMIM] database), and drugs (investigational and approved). The circular diagrams represent protein targets of drugs, and the elliptical diagram represents drugs. Approximately 500 protein drug targets are represented in the OMIM database, as depicted by the overlap of the drugome (red), research drugome (dark green), and diseaseome (pale green). Although not depicted by the black arrows in this figure, some approved drugs may have targets that are currently investigational.

Fig 2.

Biological signaling networks and signaling motifs. (A) A hypothetical biological system is represented as a protein signaling network. Each protein or molecule in the network is a node (ovals) and each interaction is an edge. The network is directional and edges are given signs, with arrows representing positive or activating interactions and plungers representing negative or inhibitory interactions. Neutral interactions (scaffolding or anchoring interactions) are represented as lines. (B) The regulatory motifs within the protein signaling network are highlighted by color or dotted/dashed boxes. A feed-forward loop (FFL) is shown in pink, a negative FFL loop is shown in blue, a positive feedback loop is shown in yellow, and a negative feedback loop is shown in green. A hub is shown in a dotted box and an island is shown in a dashed box. (C) Mutations found in disease (left) are generally null (center) or edgetic (right). Null mutations, which are caused by gene truncations either by a lack of expression or expression of a severely shortened/compromised gene product, are represented as node removals in signaling networks. Edgetic mutations are caused by point mutations or small truncations leading to the expression of a gene product that has lost an interaction with another protein, and are represented edge removal(s).

Fig 3.

Signaling intricacies determine therapeutic outcome in complex disease. (A) In complex diseases such as cancer, 1 or more activating mutations cause cellular transformation, enhanced cell growth, proliferation, and survival, leading to tumor formation and maintenance. Drugs have been developed to inhibit hyperactive signaling pathway(s) caused by the mutations in cancer, such as MEK and B-Raf inhibitors. (B) When cells are treated with a MEK inhibitor (in red), disruption of negative feedback loops (dashed lines) causes counterintuitive increases in signaling upstream of MEK (large bold arrows), such as phosphorylation of MEK by B-Raf and activation of PI3K indirectly by the RTK. (C) When B-Raf WT/Ras mutant cells are treated with B-Raf inhibitors (in red), the autoinhibitory property of B-Raf is reduced and its affinity for C-Raf is increased. This results in recruitment of B-Raf/C-Raf to Ras at the membrane, where C-Raf is activated, causing hyperactivation of MEK. When B-Raf mutant cells are treated with B-Raf inhibitors, this effect is not seen and MEK remains inhibited. **Abbreviations:** AKT, V-akt murine thymoma viral oncogene homolog; ATP, adenosine triphosphate; B-Raf, V-raf murine sarcoma viral oncogene homolog B1; C-Raf, V-raf-1 murine leukemia viral oncogene homolog 1; ERK, extracellular signal-regulated kinase; GPCR, G-protein-coupled receptor; mTORC1, mammalian target of rapamycin complex 1; MEK, mitogen-activated/extracellular signal-regulated kinase kinase; Met, methionine; mRNA, messenger RNA; PDK, 3-phosphoinositide dependent protein kinase; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; shRNA, short hairpin RNA; siRNA, small interfering RNA; Spry2, Sprouty homolog 2 (drosophila); TSC1/2, tuberous sclerosis complex tumor suppressor 1–2; WT, wild type.

Table 1

Definitions of Key Terms in Systems Pharmacology.

Term	Definition	Associated Figure
Drug target	A protein, typically an enzyme and/or transmembrane receptor, to which a drug binds and causes a change (positive or negative) in the action of.	1
Druggability	The likelihood of successfully designing a small molecule or biological molecule that can interfere with or promote the action of a given drug target.	
Genome	The overall genetic makeup of an organism; this includes coding and noncoding regions.	1
Proteome	The collection of proteins expressed by the genome.	1
Drugome	The collection of proteins that are targets of drugs.	1
Biological signaling network	Cell-signaling pathways represented as interactions between components within the system as edges and nodes. Such representations allow us to compute system properties.	2
Bipartite graph	A graph-theory term describing a network where the nodes are derived from 2 independent sets (examples cited in text are sets of drugs and drug targets or genes and diseases) and every edge connects a node from 1 group to the other group.	NA
Node	A representation of a component within a network that takes part in an interaction with another component in the network. Biological signaling network nodes can be enzymes, scaffolding proteins, ion channels, receptors, transcription factors, genes, ions, small molecules, or lipids.	2
Edge	A representation of an interaction between 2 components (nodes) within a biological signaling network. Edges represent binding reactions, gene expression, or enzymatic reactions such as post-translational modifications, molecular conversions (ie, ATP to cAMP).	2
Signaling motif	A pattern in the signaling flow within a network. Motifs include feedback loops, feed-forward loops, and bifan motifs.	2
Network redundancy	When >1 path in the signaling flow of a network leads to the same outcome.	NA

Abbreviations: ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; NA, not applicable.

Table 2

Systems Biology Studies That Elucidate Mechanisms of Pathophysiology and Drug Action.

Methodology	Description	Examples
Genomics and proteomics	Large-scale experimental analysis of gene expression, genomic or epigenetic variations; post-translational changes resulting from varying conditions; includes phosphoproteomics and chemical proteomics. Cells or tissue samples are subject to a condition (eg, drug treatment, stress, diseased versus wild type/normal) and the response is analyzed compared with a control. Expression level, genetic variation, phosphorylation status, and other post-translational modifications can be monitored.	16·46·48 21·49·50
Large-scale molecular screening and drug structure-target analyses	Experimental and computational methods uncover unknown targets and/or promiscuity of drugs. These analyses can allow for further characterization the targets that are inhibited by common drugs, or vice versa.	6·23·42·49·51·52·53
shRNA/siRNA screens	A high-throughput version of silencing a single gene and analyzing the effect in cells. Resulting information can explain/predict the function of a protein in disease or the effect of inhibiting the protein as a means of drug-target validation.	54·57 58·59
Network analysis and computational methods	The identification and understanding of networks surrounding a drug target, often using published data from high-throughput studies (such as those listed above) or databases.	8·9·20·22·60·61

Abbreviations: shRNA, short hairpin RNA; SILAC, stable isotope labeling by amino acids in cell culture; siRNA, small interfering RNA.

Table 3Currently Approved Targeted Therapies for the Treatment of Cancer.^{62–65}

Therapy (Trade Name) Company	Therapy (Primary in bold)	Disease
Imatinib (Gleevec) Novartis	Abl , c-Kit, PDGFR, CSF1R, NTRK1, DDR1	CML, ALL, CEL, GIST
Dasatinib Sprycel	Src, Abl , c-Kit, PDGFR	CML (imatinib resistant), ALL
Nilotinib (Tasigna) Novartis	Bcr-Abl, Abl , PDGFR, c-KIT, DDR1	CML (imatinib resistant)
Erlotinib (Tarceva) OSI/Genentech/Roche	EGFR, ErbB2	NSCLC, pancreatic cancer
Gefitinib (Iressa) AstraZeneca	EGFR , ErbB2, ErbB4	NSCLC
Sorafenib (Nexavar) Bayer/Onyx	B-Raf , c-KIT, PDGFR, VEGFR	Renal cancer
Sunitinib (Sutent) Pfizer	c-KIT, PDGFR, VEGFR, KIT, RET, CSF1R	Renal cancer, GIST (imatinib resistant)
Lapatinib (Tykerb) GlaxoSmithKline	ErbB2	Breast cancer
Pazopanib (Votrient) GlaxoSmithKline	VEGFR, PDGFR, c-Kit	Renal cancer
Trastuzumab (Herceptin) Genentech	ErbB2	Breast cancer

Abbreviations: ALL, acute lymphoblastic leukemia; CEL, chronic eosinophilic leukemia; CML, chronic myelogenous leukemia; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer.