Chinese Journal of Cancer

Review

Network systems biology for targeted cancer therapies

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Abstract

The era of targeted cancer therapies has arrived. However, due to the complexity of biological systems, the current progress is far from enough. From biological network modeling to structural/dynamic network analysis, network systems biology provides unique insight into the potential mechanisms underlying the growth and progression of cancer cells. It has also introduced great changes into the research paradigm of cancer-associated drug discovery and drug resistance.

Key words Targeted cancer therapy, network systems biology, network modeling, network analysis, drug discovery, drug resistance

Targeted cancer therapies refer to a new generation of anti-cancer drugs designed to interfere with specific molecular targets (typically proteins) that are believed to be critical in tumor growth or progression[1]. By precisely attacking these cancer-causing molecules, targeted cancer therapies can slow down the growth and proliferation of tumor cells, induce apoptosis in these cells or trigger the immune system to recognize and destroy these cells. Due to their fantastic efficacy, targeted cancer therapies have been accepted as a great success in treating many types of cancer[2]. However, along with the deepening of our comprehension of cancer as well as the soaring amount of the available high-throughput "-omics" data, the development of targeted cancer therapies is facing great challenges. The major challenges are the drug target prediction and the solution for drug resistance.

To a great extent, the efficacy of targeted cancer therapies depends on the molecules selected as drug targets. The molecules execute their function through various types of interactions. Considering that biological systems are intrinsically complex and that our knowledge on how tumor cells grow and proliferate is limited, the development of targeted cancer therapies, especially the identification of potential drug targets, therefore requires a more detailed understanding of how the modification of molecules and the transduction of biological signal flows drive the disease.

Another problem is drug resistance in cells. The resistance mechanisms are pleiotropic but analogous to those with whom "classical" cytotoxic anti-cancer drugs have struggled, including enhanced activity of drug pumps[3,4], modulation of cell death pathways[5,6], alteration and repair of target molecules[7], redundancy of biological regulations[8], and other various, less common mechanisms[9] (detailed overviews in Reference [10]). Together, these mechanisms form a complex network of cellular pathways that mediate an individual multi-drug resistance (MDR) phenotype.

The widespread emergence of systems biology meets timely with the increasing urge for developing targeted therapies. Using the overwhelming '-omics' data, systems biology manages to quantify all the molecular elements of biological systems, healthy or diseased, to assess their interactions and integrate them into various graphical network models, and then to explore these networks to reveal the underlying biological mechanisms[11-14]. In systems biology, not only are miscellaneous databases (Table 1) and platforms (such as Cytoscape[15] and its plug-in corps) developed to pave the way for network modeling, but network analysis algorithms are also proposed to study the disease-associated molecules and pathways from a systematic and network-based view. The concept of "network" plays an irreplaceable role in the systems biology in the post-genomic era. To highlight this, we denominate the network-based part of systems biology as "network

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doi: 10.5732/cjc.011.10282

Table 1. Network data resources

Category	Database	Website	Contents	Reference(s)
Protein databases	UniProtKB	http://www.uniprot.org/	The central collection of functional information on proteins, with accurate, consistent, and rich annotation. It has tremendous and extensive influence in the post-genomic era.	[67,68]
	Nextprot	http://www.nextprot.org/	A very novel but promising knowledge resource centered on human proteins. Incorporates all human-centric protein data in UniProtKB/Swiss-Prot as well as carefully selected and filtered high-throughput data pertinent to human proteins.	
Protein-protein interaction databases	STRING	http://string-db.org/	Contains known and predicted physical and functional protein interactions derived from different sources. It currently covers 5 214 234 proteins from 1133 organisms.	[69,70]
	DIP	http://dip.doe-mbi.ucla.edu/	Contains protein interactions derived from a variety of sources but curated both manually and automatically using computational approaches. It supports search by proteins, sequences, motifs, articles, and pathways.	[71]
Pathway databases	KEGG	http://www.kegg.com/	Integrates genomic, chemical, and systemic functional information. Gene catalogs in the completely sequenced genomes are particularly linked to higher-level systemic functions of the cell, the organism, and the ecosystem.	[72]
	Reactome	http://www.reactome.org/	Comprises 4166 human reactions organized into 1131 pathways involving 5503 proteins encoded by 5078 human genes. Data is manually curated and peer-reviewed by biologists.	[73,74]
Drug-target databases	Drugbank	http://www.drugbank.ca/	Combines detailed drug data with comprehensive drug targets. To date, it contains 6829 drug entries and supports search by pathway.	[75–77]
	STITCH	http://stitch.embl.de/	Designed to explore known and predicted interactions of chemicals and proteins. It contains interactions for over 74 000 small molecules and over 2.5 million proteins in 630 organisms.	[78,79]
	PROMISCUOUS	http://bioinformatics.charite.de/promiscuous	Contains data of drugs, proteins, and side effects, as well as relations between them. It has three search methods: by drug, by target, and by pathway.	[80]

More databases are summarized in References [45,81,82].

systems biology" in the remainder of this review.

Herein, we first present an outline of network systems biology, focusing on the expatiation of the two types of network analysis strategies that are often used. Then, in the next two sections, we review how drug target prediction and drug resistance prevention, the two points of interest in the research of targeted therapies, respectively benefit from network systems biology. Finally, we conclude with a summary and a brief outlook.

Network Systems Biology

In network systems biology, biological systems are first modeled into networks for different research purposes and then are explored with the use of network-based strategies.

In the network modeling stage, protein-protein interaction networks, transcriptional regulatory networks[16], signaling transduction networks[17-19], and metabolic networks[20] are often used. In protein-protein interaction networks, nodes represent proteins and edges represent associations between proteins. The protein associations can be abstract relations between proteins rather than concrete and direct/indirect protein interactions, such as protein binding. For example, the polypharmacology relationships, that is, the links between a pair of proteins that share a certain number of compounds, can be regarded as the edges, and with this assumption the drug-associated protein system can be modeled into the protein-protein interaction network[21]. In different research contexts, chemokine networks[22], drug-target networks[23], disease-gene networks[24], and others are also frequently used.

The modeled networks are normally analyzed using two types of strategies [18]: static network analyses and dynamic network analyses (Figure 1). Static network analyses, also known as structural network analyses, are often used to explore network properties by computing the time-invariant topological properties of the network. These topological properties can be divided into two categories: global topological properties and individual topological properties (Table 2). Global topological properties include node degree distribution, path length, clustering coefficient, network diameter, and others. From different angles, these properties help to understand how the modeled biological system behaves as a whole when random errors (e.g. gene mutation) or malicious attacks (e.g. targeted treatment) happen in either a provisional or a persistent way [25,26]. Individual topological properties refer to how important the individual elements, such as specific nodes, edges, network motifs [27], and network modules [28], are in the current network context. These properties are presented as different topological centralities, such as degree centrality, closeness centrality, betweenness centrality, bridging centrality, and the like. They respectively reveal distinct importance the elements manifest and the different ways in which they participate in maintaining the network stability.

Compared to static network analyses, dynamic network analyses are considered to be able to reveal the time-variant properties or predict the dynamic changes of the network [18]. Depending on whether the associated kinetic parameters are known or not, dynamic network analysis approaches can also be classified into two groups. In the case that the parameters are sufficient and known, approaches such as ordinary differential equations (ODE)[29], flux balance analysis (FBA)[30], and those based on the control theory [31] can be carried out. For example, with the obtained timescales and kinetic parameters, the response of special signaling activities can be estimated using ODE, beginning with listing all biochemical transformations and thereby providing a kinetic scheme of signaling pathways[32].

Typically, numerical values for kinetic parameters are difficult to obtain, so ODE and control theory-based dynamic analyses are limited to stoichiometric reconstructions of small-scale cellular networks. Thus, when a priori kinetics are insufficient, logic-based models and Petri net-based models [33-37] are normally used. Logic-based models include Boolean logic models, multi-state discrete models, and fuzzy logic models [38,39]. Boolean logic models assume that all agents in a signaling network are either "on" (state 1) or "off" (state 0), whereas multi-state discrete models specify additional levels between 0 and 1, and fuzzy logic models allow for continuous agent states. With these assumptions, logic-based models have been widely used in estimating the dynamic characteristics of biological networks [40-42]. Petri net-based models, such as the signaling Petri net (SPN)[37] and the colored Petri net (CPN)[33], simulate the network dynamic behavior based on the Petri net theory. Compared to logic-based models, Petri net-based

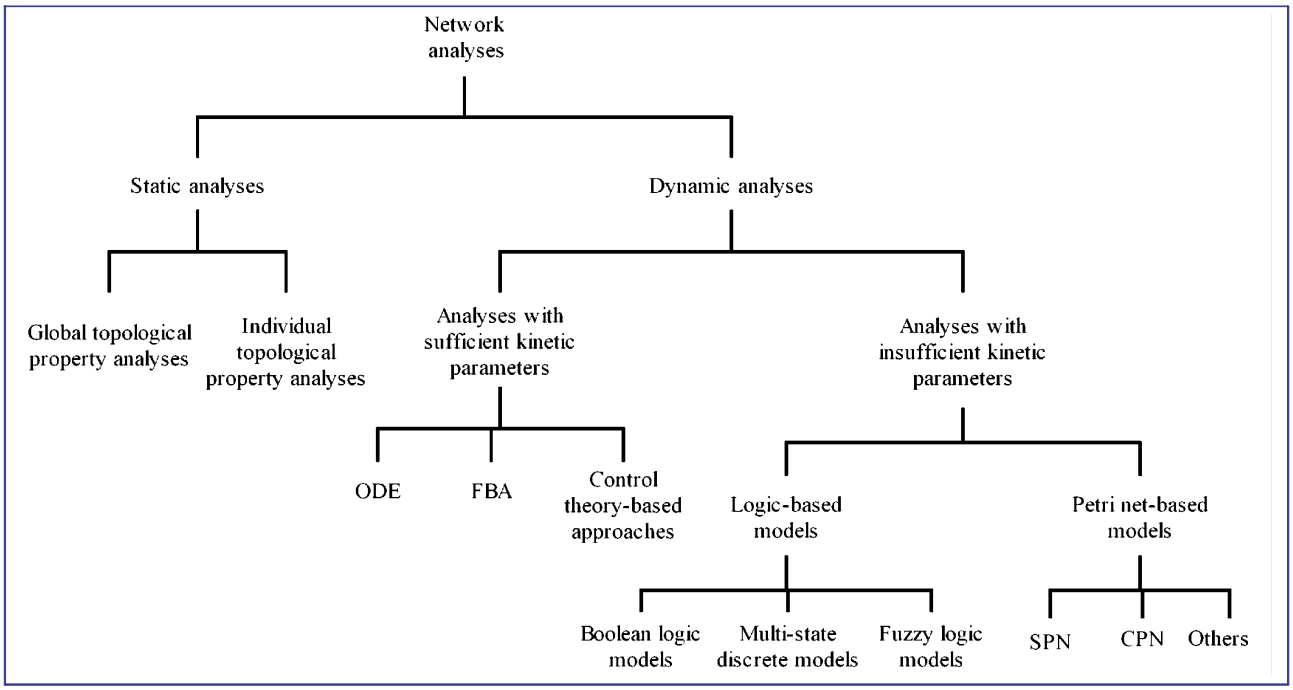

Figure 1. **The relational tree of different network analysis strategies.** ODE, ordinary differential equations; FBA, flux balance analysis; SPN, signaling Petri net; CPN, colored Petri net.

Table 2. Topological properties and their significance

Category	Property	Significance	Reference(s)
Global	Degree distribution	Defined as the probability distribution of degree of all the nodes. Networks with power-law degree distribution are supposed to be scale-free.	[83,84]
	Average path length	Defined as the arithmetic mean of all the path lengths in the network.	[83]
	Clustering coefficient	Defined as the arithmetic mean of clustering coefficients of all individual nodes.	[83]
	Network diameter	Defined as the maximum path required to connect any two nodes.	[83]
Individual	Degree centrality	Mathematically equals the node degree, which is defined as the number of links incident upon the given node. The higher the degree centrality of the given node, the more associated nodes are influenced by the change of this node and thus, the more critical it is.	[84]
	Closeness centrality	Defined as the mean length of all the shortest paths between the given node and all the other nodes reachable from it. The lower the closeness centrality of the given node, the sooner the influence that arises from the change of the given node can spread to all the reachable nodes and thus, the more critical it is.	[84]
	Betweenness centrality	Defined as the proportion of all shortest paths between node pairs in a network passing through the measured node. The higher the betweenness centrality of the given node is, the higher the number of pairs of nodes it mediates and thus, the more critical it is.	[84]
	Bridging centrality	Defined as the product of the rank of the given node in random betweenness and the rank in bridging coefficient. The nodes with high bridging centrality are critical because they locate between and connect modular subregions in the network.	[85]

More databases are summarized in References [45,81,82].

models are more flexible on the definition of system state and the description of signaling transmission, and have also been very successful in dynamically simulating signaling mechanisms and transcriptional activities[43].

Network-based Drug Target Prediction

One main application of network analyses is to predict or identify potential targets for the development of targeted therapies. The general workflow begins from integrating the available "-omic" data into some specific network models, and then using proper network analysis strategies, especially static network analyses, to explore the properties of the modeled network to identify proteins with the potential to be candidate drug targets.

A very comprehensive and successful example of this process has been described by Pujana *et al*.[44]. They used the network strategy in cancer-associated gene prediction and identified one gene that encodes hyaluronan-mediated motility receptor (HMMR) and directly interacts with the well-known breast cancer-associated gene breast cancer 1, early onset (*BRCA1*). Their work started from collecting the data around four known breast cancer-associated genes: *BRCA1*; breast cancer 2, early onset (*BRCA2*); ataxia telangiectasia mutated (*ATM*); and checkpoint kinase 2 (*CHEK2*). Then, they combined gene expression profiling data with functional genomics and proteomics data derived from various species to model the heterogeneous protein network. From this network, they "extracted" a BRCA1-centred network (BCN) and compared it with a same-scale random network. The comparison indicated the potential function of the clustered BCN components. After that, they looked for new potential connections among different functional components of the BCN. According to the computed co-expression correlation value (inversely proportional to the edge length) of the nodes in the BCN, they focused the prediction on HMMR, the gene that is relative to *BRCA1* but plays an important role in linking together the different functional parts of the BCN. These findings have since been validated, with two case-control studies of incident breast cancer suggesting that HMMR could indicate susceptibility for breast cancer within diverse human populations[44].

This work is a remarkable demonstration of the use of network-based strategies for drug target prediction. This approach not only identifies cancer-associated genes that have the potential to be drug targets of the next generation, but also provides a detailed roadmap of how to predict and identify cancer-associated genes or proteins in the network context. Moreover, this work provides a successful strategy for integrating a vast variety of "-omics" data to reconstruct disease-associated protein networks, and it supports the

feasibility of using gene homology and gene ontology to mine proteins and protein associations that are unclear in humans but have been well studied in other model organisms.

In addition to protein networks, other types of biological networks, such as metabolic networks, transcriptional regulation networks, and signaling networks, are also frequently used to model the disease-associated *in vivo* environment and contribute to the network-based drug target prediction[45].

Metabolic networks focus on the intricate exchange of chemical groups and redox potentials through a set of carrier molecules, a process in which enzymes play the leading role. For this type of network, mathematical analyses are suitable to be carried out in a relatively precise way, in line with the stoichiometric matrix. Therefore, in addition to the general topological static analyses that indicate the error and attack tolerance of metabolic networks from a global view[25], powerful kinetic models such as ODE or FBA can be set up to trace the network response against changes in enzyme activity and compound concentration. Examples include the model developed for predicting the onset of avascular tumor growth among cells in response to the loss of p53 function[46], as well as the model for developing hypoxia-inducible factor-1α (HIF-1α)-based therapies[47].

Regulatory networks, especially transcriptional regulatory networks, are usually concerned with the interplay of transcription factors. Abnormal activity of transcription factors is associated with the change of critical gene expression or the redirection of signaling cascades[16]. Modeling regulatory networks and recognizing their structures help to clarify the functional position of target-associated transcription factors and yield candidates for potential drug targets[48-50]. As a successful example, Bai *et al.*[49] used differentially expressed gene data and transcription factor-gene relationship data to construct gene regulatory networks, and then executed a transcriptome network analysis method to select candidate genes for squamous lung cancer. The results showed that 5 out of 6 selected candidates were purportedly involved in lung cancer.

Signal transduction networks model the transduction process of signals in pathways. Signals are not only linearly transmitted, but also processed through signal integration, cross-activation, and positive or negative feedback in a time sequence. Based on this knowledge, overall static analyses that shed light on the importance of different molecules in signal transduction networks are often used. In addition, ODEs, logic-based models, and Petri net-based approaches are also often exploited to represent concrete signaling systems because they enable prediction of the effects of, for example, apoptosis and DNA damage[51] as well as various ligand stimulations[41].

Network-based Research on Drug Resistance

In a biological system, the roles even a single molecule plays in different contexts can be complex, and this is one primary reason for drug resistance. For example, the inappropriate expression of the multidrug resistance (*MDR1*) gene encoding P-glycoprotein (P-gp) is reported to cause a drug resistant phenotype in an imatinib-treated leukemia cell line[52]. Because imatinib is a substrate for breast cancer resistance protein (BCRP) but not for multidrug resistance-associated protein 1 (MRP1) and because it has also been demonstrated to be an inhibitor of BCRP, imatinib has the potential to modulate the pharmacology of other drugs that are BCRP substrates[53]. Figure 2 displays the network constructed from these drug resistance-associated proteins. This network not only illustrates the interactions among these drug resistance-associated proteins, but also shows how the network facilitates the process of hunting for the proteins that are possibly involved in this MDR phenotype and how important they could be.

Other causes of drug resistance include redundant signaling pathways and compensatory pathways, as well as loss of feedback loops that are critical to the stabilization of signaling systems. Most instances of drug resistance against targeted therapies are associated with the cell signaling process[54], thus signaling networks or protein-protein interaction networks that model signaling pathways are often adopted. Disease-perturbed signaling networks can be either reconstructed from the available public pathway databases such as KEGG and Reactome or reversely engineered from gene expression data[55]. The former reconstruction method is limited by a small amount of pathway data, whereas the latter bears a considerable computational complexity.

With reconstructed disease-associated signaling networks, both static and dynamic network analysis strategies can be applied to explore the details of how the resistance is developed by the cells. For example, resistance to trastuzumab is observed in treating receptor tyrosine-protein kinase erbB-2 (ERBB2)-overexpressed breast cancer patients[56]. The major cause is purportedly that cancer cells engage compensatory pathways to overcome cell cycle arrest despite inactivation of the ERBB2 receptor. To gain insight into the potential mechanisms underlying this process, Sahin *et al.*[40] employed a Boolean logic model to represent the regulatory interactions of G_1/S transition of the cell cycle, and simulated the loss of function of one or multiple proteins. Tested experimentally, these simulation results show that combinatorial targeting of ERBB receptors or of key signaling intermediates has no potential for treatment of *de novo* trastuzumab-resistant cells. Instead, c-MYC perturbation might be responsible for cell

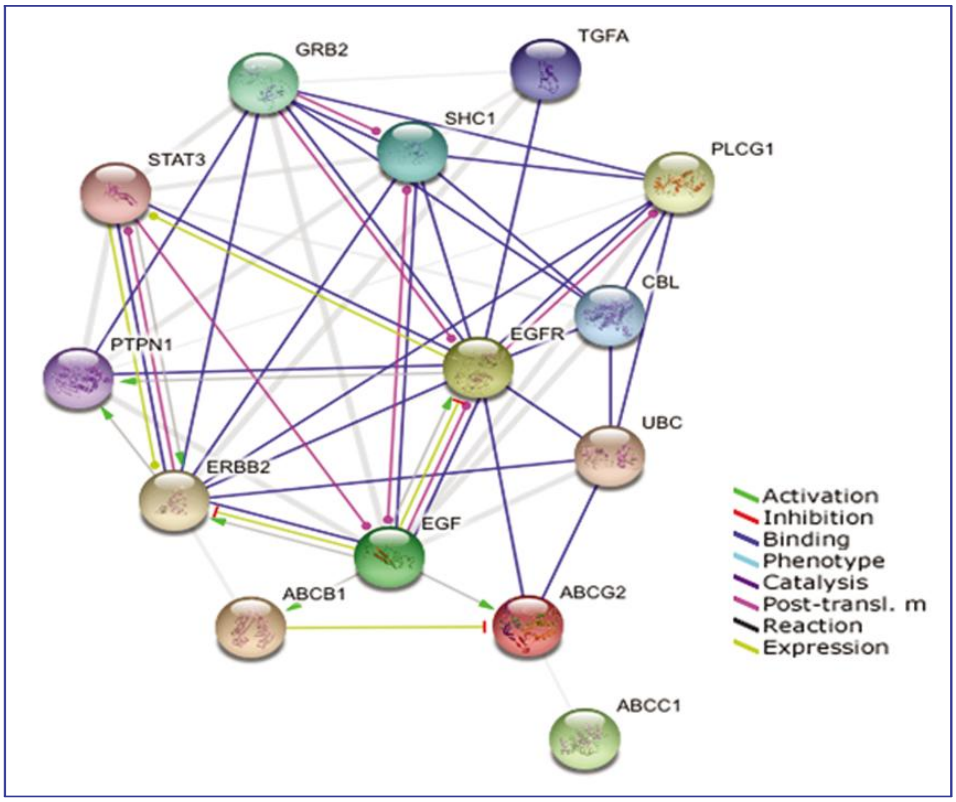

Figure 2. **Demonstration of protein-protein interaction network of epidermal growth factor receptor (EGFR)-associated multidrug resistance, collected and displayed by STRING[66].** This demonstration was run using MDR1/P-gp (ABCB1), MRP1 (ABCC1), BCRP (ABCG2), and EGFR as the input to search the STRING database for protein associations. The results were expanded to the current network by setting the required confidence score as 0.400. Different types of protein associations are marked in different colors, and the directions of directed associations are marked with different arrow shape. Uncertain associations according to STRING are shown in gray. From this network, one could roughly tell the importance of these proteins from the degree they possess.

sensitivity or resistance to trastuzumab.

According to differential expression profile analysis, therapy resistance is associated with over-expression of a unique set of proteins, which reflect potential mechanisms of reactivation[57]. These proteins (or protein families) can be "switches" that divert the signal to compensatory pathways[58]. In the context of protein-protein interaction or signaling networks, the proteins acting as switches are possibly the highly important nodes, such as the "party" or "date" hubs that have pleiotropic functions across the network, or the bridging nodes that help in exchanging signals among network modules[59,60]. Therefore, computational static network analyses, such as ranking nodes by their importance, decomposing the network into functional modules, and comparing networks of the same system but in different states[61] (for example, pre- or post-resistance), can suggest the potential factors that perturb the efficacy of targeted therapies. These analyses can lead to more reasonable cancer treatments, like combination therapies[62], to help to eliminate acquired drug resistance.

Outlook

For many years, clinical biologists have suffered from not having a comprehensive roadmap on the underlying and complex mechanisms of cancer. Now, however, thanks to the emergence of high-throughput "-omics" data and the rapid advances of systems biology in this post-genomic era, researchers have started to consider cancer treatment from a global perspective. The concept of network systems biology not only enables the discovery of potential drug targets by making the most of known information on cancer, but also explicates why current targeted therapies, the so-called "magic bullets", cannot bypass the various kinds of resistance developed by cells.

As discussed in previous sections, network systems biology has greatly changed the paradigm of developing targeted cancer therapies. It continues making our understanding of cancer multi-dimentional and more comprehensive. It also changes the traditional experiential medical treatment into the so-called "network pharmacology"[63]. In addition to more reasonable molecule-targeted therapies, multi-target drugs[64], personalized bespoke medicines[65], pathway-targeted therapies[66], and so on, whatever network systems biology is dedicated to bringing us in the way of fighting cancer, we look forward to, with hope.

Acknowledgment

The author thanks Prof. Jian-Nan Feng, Dr. Jing Geng, and especially Prof. Hui Peng for the help on this

paper. The author also thanks the anonymous reviewers for their valuable comments and revision to improve the manuscript. This work is funded by the National Natural Science Foundation of China (31100961, 81173082, and 30873083).

Received: 2011-07-12; revised: 2011-09-30; accepted: 2011-10-10.

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