Using gene networks to drug target identification

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Abstract

The complete genome sequences have provided a plethora of potential drug targets. Gene network technique holds the promise of providing a conceptual framework for analysis of the profusion of biological data being generated on potential drug targets and providing insights to understand the biological regulatory mechanisms in diseases, which are playing an increasingly important role in searching for novel drug targets from the information contained in genomics. In this paper, we discuss some of the network-based approaches for identifying drug targets, with the emphasis on the gene network strategy. In addition, some of the relevant data resources and computational tools are given.

1 Introduction

The classical progression of the pharmaceutical discovery process goes from drug target to lead compound to drug. The ability to discover novel therapeutic targets for further research is the first critical step in this process. It is reported that approximately 483 drug targets account for nearly all drugs currently on the market (45% receptors, 28% enzymes, 5% ion channels, and 2% nuclear receptors) [1]. The complete sequencing of the human genome has revealed thousands of potential drug targets, which indicates the huge potential for drug target discovery and will have a significant impact on the process of drug development [2-4]. However, currently most new drugs that are approved by the regulatory authorities modulate protein targets for which marketed drugs already exist [5]. Therefore, one major hurdle for drug development is still the rapid and accurate identification of drug targets with true potential. Gene network technique holds the promise of providing a conceptual framework for analyzing the profusion of biological data being generated on potential drug targets and providing insights to understand the biological regulatory mechanisms in diseases, which has been playing an increasingly important role in searching for novel drug targets from the information contained in genomics [6,7]. Here, we discuss the network-based *silico* methods and data resources for the identification of drug targets.

2 Methods for drug target identification

2.1 What may be potential drug targets?

Drug targets are membrane or cellular receptors or other molecules that are pivotally involved in disease processes. From a pharmacological viewpoint, a drug target is either inhibited or activated by drug molecules (e.g. small organic molecules, antibodies, therapeutic proteins). Drug molecules can physically attach to a drug target, triggering a cascade of intracellular biochemical reactions, followed by a cellular reaction. Potential drug targets can include genes that are differentially expressed between individuals who are and are not in need of

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treatment for a particular disease or condition, genes that are differentially expressed when an individual is exposed to a drug known to alleviate or exacerbate the symptoms of interest, and genes that are co-expressed with other genes presumed to be involved in the systems and pathways under study. Any gene falling into one of these categories may be a gene for which manipulation of its expression might affect disease or symptom progression [8]. In summary, good drug targets are potent and specific, that is, they must have strong effects on a specific biological pathway and minimal effects on all other pathways.

2.2 Data resources for drug target identification

Drug target identification involves acquiring a molecular level understanding of a specific disease state and includes analysis of gene sequences, protein structures, protein interactions and metabolic pathways [9]. The ultimate goal of the process is to discover a suitable target whose biological activity can be directly linked to a pathological process. In the age of genomics, discovery of novel drug targets needs to incorporate and integrate different sources of data including gene expression data, gene sequence data, gene polymorphism data and so on. Many public biological databases are warehousing and providing a great amount of functional information for drug discovery. Table 1 lists some relevant databases for drug target identification, yet one of the most important information is the human genome itself and associated annotations. In addition, the public data infrastructure is as important as the data and includes algorithms for sequence homology searching, transcription data analysis, protein structure prediction and so on [10]. Integrating existing data from public databases to create systematic analysis architecture will be helpful for inferring the underlying interaction of genes and gaining insights about the pathway structures with which drug targets interact.

Database	Access	Contents	Refs
ArrayExpress	http://www.ebi.ac.uk/Microarray/ArrayExpress/arrayexpress	Public repository of microarray data	[11]
BIND	http://bind.ca	The biomolecular interaction network database	[12]
GeneNet	http://wwwmgs.bionet.nsc.ru/systems/MGL/GeneNet/	Gene network database	[13]
GEO	http://www.ncbi.nlm.nih.gov/geo/	Gene expression omnibus	[14]
GPCRDB	http://www.gpcr.org/7tm/	GPCR database	[15]
KEGG	http://www.genome.ad.jp/kegg/	Kyoto encyclopedia of genes and genomes	[16]
KinG	http://hodgkin.mbu.iisc.ernet.in/~king	Protein kinases database	[17]
LGICdb	http://www.ebi.ac.uk/compneur-srv/LGICdb	Database of ligand-gated ion channels	[18]
MEROPS	http://www.merops.ac.uk	Peptidase database	[19]
NucleaRDB	http://www.receptors.org/NR/	Nuclear receptors database	[20]
NUREBASE	http://www.ens-lyon.fr/LBMC/laudet/nurebase	Database of nuclear hormone receptor	[21]
OMIM	http://ww3.ncbi.nlm.nih.gov/Omim/	Online mendelian inheritance in man	[22]
SMD	http://genome-www5.stanford.edu/Microarray/SMD/	Stanford microarray database	[23]
TRMP	http://xin.cz3.nus.edu.sg/group/trmp/trmp.asp	Therapeutically relevant multiple pathways database	[24]
TTD	http://xin.cz3.nus.edu.sg/group/cjttd/ttd.asp	Therapeutic target database	[25]
PIM	http://proteome.wayne.edu/PIMdb.html	Protein interactions maps database	[26]

Table 1. List of some relevant databases for drug target identification.

2.3 The network-based strategy for drug target identification

With the development of bioinformatics, a number of computational techniques have been used to search for novel drug targets from the information contained in genomics. The network-based strategy for drug target identification attempts to reconstruct endogenous metabolic, regulatory and signaling networks with which potential drug targets interact. Once having these information provided by gene networks or protein networks, the interaction relationships between potential drug targets could be explicitly revealed, so it could be easily determined which one of these potential drug targets is most proper, or the scope of selecting candidate drug targets could be narrowed down to a great extent [27-29], for example, if a potential drug target participates in many biological pathways, the inhibition of this target may interfere with many activities associated with those pathways, and therefore, may not be a good candidate for drug target. Along with the development of microarray technology, large volume of gene expression or protein expression data have been produced, and there have been considerable models proposed to infer gene networks or protein networks from these data.

2.3.1 Gene network strategy for drug target identification

The molecular interactions of genes and gene products underlie fundamental questions of biology. Genetic interactions are central to the understanding of molecular structure and function, cellular metabolism, and response of organisms to their environments. If such interaction patterns can be measured for various kinds of tissues and the corresponding data can be interpreted, potential benefits are obvious for the identification of candidate drug targets. It has already been demonstrated that it is possible to infer a predictive model of a genetic network by time-series gene expression data [30] or steady-state gene expression data of gene knockout [31]. Using the inferred model, useful predictions can be made by mathematical analysis and computer simulations. Recently several computational methods have been proposed to reconstruct gene networks, such as Boolean networks [32], differential equation models [33] and Bayesian networks [34]. These quantitative approaches can be applied to natural gene networks and used to generate a more comprehensive understanding of cellular regulation, discover the underlying gene regulatory mechanisms and reveal the interactions between drugs and the drug targets in cells.

- *Data requirement*

To discover genes of pharmaceutical interest, various microarray data, such as drug response expression data, time-course expression data and steady-state expression data of gene knockout, could be used. With the drug response expression data, a set of drug-affected genes could be collected by differential expression data analysis. However, this set is usually too coarse to determine the effective drug targets. So further gene network information that can reveal the interactions between genes are necessary to facilitate the drug targets identification. The large-scale gene expression profiles measured in time series or gene deletion experiments are invaluable sources for identifying gene regulatory networks, which can give more meaningful information about biological processes. Noticeably, the time-course microarray data can give more insights into the causality and regulation of cells [35]. The great advantage of investigating time-series gene expression data is that gene networks can be readily derived from the data using simple dynamic models. Imoto *et al.* [34] demonstrated this process of drug targets identification by using two kinds of cDNA microarray data mentioned above, one is the microarray data obtained by gene disruptions (gene knock out) and used to constructed the gene networks, the other is the drug response expression data used to collect drug affected genes.

- *Models for estimating gene networks*

In the gene network-based strategy for drug target identification, the estimated gene networks play an important role. The reconstruction of gene networks from gene-expression data is gaining popularity as methods improve and as more data become available. In order to draw meaningful inferences from gene expression data and find regulatory relationships between genes, it is important that each gene is surveyed under several different conditions, preferably in the form of expression time series. Such data sets may be analyzed using a range of methods with increasing depth of inference, such as cluster analysis [36,37], correlation statistics analysis [38,39], weight matrices [40,41], neural networks [42], genetic algorithms [43], and supervised learning algorithms [44].

For the purpose of capturing the interrelated regulatory mechanisms between genes, several genetic network models have been proposed, such as Boolean networks [45,46], differential equations [47,48], and Bayesian networks [34,49-53], which use expression data to trace genetic regulatory interactions. Of these mentioned models, Bayesian networks are promising for learning gene regulatory networks from observed expression data [30]. The inferences of genetic networks provide insights into the underlying gene interactions and gene regulation of metabolic pathways in the living organisms. With the development of gene regulatory network modeling, many computational tools for the reconstruct gene networks have been developed. These tools enable a user to readily reconstruct genetic networks based on microarray data without having intimate knowledge of the mathematical models, and could be conveniently used for identification of potential targets for therapeutic [54]. Table 2 lists some relevant model-based computational tools that are publicly available.

Tools	Access	Contents	Refs
ASIAN	http://eureka.ims.u-tokyo.ac.jp/asian	Website for network inference	[55]
BioMiner	http://www.zbi.uni-saarland.de/chair/projects/BioMiner	System for analyzing and visualizing biochemical pathways and networks	[56]
DBmcmc	http://www.bioss.sari.ac.uk/~dirk/software/DBmcmc/	Tool of inferring dynamic bayesian networks	[57]
GNA	http://www-helix.inrialpes.fr/gna	Tool for the modeling and simulation of genetic regulatory networks	[58]
GenMAPP	http://www.GenMAPP.org	Tool for viewing and analyzing microarray data on biological pathways	[59]
GeneNetwork	http://genenetwork.sbl.bc.sinica.edu.tw/	Tool for reconstruction of genetic networks	[60]
GenePath	http://genepath.org	Tool for automated construction of genetic networks from mutant data	[61]
GSCope	http://gscope.gsc.riken.go.jp/	Tool for interactive modeling and analyzing biological pathways	[62]
MetNet3D	http://www.vrac.iastate.edu/research/sites/metnet	3D virtual reality system for network modeling	[63]
Path Finder	http://bibiserv.techfak.uni-bielefeld.de/pathfinder/	Tool for biochemical pathways reconstruction and dynamic visualization	[64]
Pathway Miner	http://www.biorag.org/pathway.html	Extracting gene association networks from molecular pathways	[65]
TFBScluster	http://hscl.cimr.cam.ac.uk/TFBScluster_genome_34.html	Web-based tool of transcriptional regulatory networks	[66]
ToPNet	http://www.biosolveit.de/ToPNet/	Tool for joint analysis of biological networks and expression data	[67]
VisANT	http://visant.bu.edu	Integrative platform for network/pathway analysis	[68]

Table 2. List of some relevant computational tools for gene network identification.

- *Determining novel drug targets from network structures*

Drug target identification involves acquiring a molecular level understanding of the function of drug targets. On the molecular level, function is manifested in the behaviour of complex networks. It is necessary to know the cellular context of the drug target and the impact of its inhibition or activation on multiple signaling pathways. Graphical models are often used to describe genetic networks. Generally, a gene network could be presented in a directed graph, in which nodes indicate genes and edges represent regulations between genes (e.g. activation or suppression). Analyzing the network structures of large-scale interrogation of cellular processes holds promise for the identification of essential mediators of signal transduction pathways and potential drug targets. In order to find proper candidate target genes, one needs biological knowledge of the pathways underlying the disease process. So the study of biochemical pathways is the focus of numerous researchers. However, owing to the complexity of pathway structures, many potential drug targets turned out worthless because the pathways in which they participate were more complex than expected. A promising strategy is to examine the functionality of different genes in the network and observe the connectivity of different functional domains.

Some researchers have implemented this gene network-based strategy for drug target identification [34]. First, using the gene expression data obtained from expression experiments of several dose and time responses to the drug, those genes affected by the drug (drug-affected genes) could be identified by fold-change analysis [69] or virtual gene technique [34]. Because there is no guarantee that genes most affected by the drug are the genes that were "drugged" by the drug agent, nor is there any guarantee that the drugged target represents the most biologically available and advantageous molecular target for intervention with new drugs, they further searched the most proper drug target genes upstream of the drug-affected genes in a regulatory network. Using gene expression profiles obtained from 120 gene disruptions, they employed a method based on Bayesian network model to construct a gene network. Then, by exploring the gene network, they found the “druggable genes”, namely drug targets regulating the drug-affected genes most strongly, and a novel drug target gene was identified and validated.

2.3.2 Protein interaction network strategy for drug target identification

Proteins are the principal targets of drug discovery. Knowing what proteins are expressed and how is therefore the first step to generating value from the knowledge of the human genome [70]. Proteomics has unique and significant advantages as an important complement to a genomics approach. High-throughput proteomics, identifying potentially hundreds to thousands of protein expression changes in model systems following perturbation by drug treatment or disease, lends itself particularly well to target identification in drug discovery [71,72]. Protein-protein interaction is the basis of drug target identification [73]. Protein interaction maps can reveal novel pathways and functional complexes, allowing ‘guilt by association’ annotation of uncharacterized proteins. Once the pathways are mapped, these need to be analyzed and validated functionally in a biological model. It is possible that other proteins operating in the same pathway as a known drug target could also represent appropriate drug targets. Recent analyses of network properties of protein-protein interactions and of metabolic maps have provided some insights into the structure of these networks. So identifying protein-protein interactions can provide insights into the function of important genes, elucidate relevant pathways, and facilitate the identification of potential drug targets. Powerful bioinformatics software enables rapid interpretation of protein-protein interactions, accelerating functional assignment and drug target discovery.

3 Discussion

No matter whether the number of actual drug targets is correct or not, the available data strongly suggest that the present number of known and well-validated drug targets is still relatively small. Bioinformatics is making practical contributions in identifying large number of potential drug targets, however, target validation efforts are required to link them to the aetiology of known diseases and/or to demonstrate that the novel targets have relevant therapeutic potential. The biochemical pathways put a drug target into context: one can chart those in which a target is seen, and thus make educated guesses about the effects that blocking the target are likely to have. Further, more complete knowledge of biological pathways should be used to gain clues for potential target proteins [35,74].

Despite the promising results obtained in the different tests carried out by this strategy, there are several potential problems in applications to drug target identification and validation. First, it is yet unclear if the currently available genomic databases, coupled with newly developed computational algorithms, can offer sufficient information for automated *in silico* drug target identification. Naturally, the sole use of microarray data has limitations on gene network estimation. For improving the biological accuracy of estimated gene networks, other biological information such as sequence information on promoter regions and protein-protein interactions should be integrated. Secondly, as real biological processes are often condition specific, and gene expression data tend to be noisy and often plagued by outliers, it is important to take “conditions” or “environments” into account. The problem of capturing long-run network behavior for large-size networks is difficult owing to the exponential increase of the state spaces. Thirdly, an increasing population of bioinformatics tools and the lack of an integrated and systematized interface for their selection and utilization is becoming widely acknowledged. Last and perhaps more important, understanding how a target protein works in the context of cellular pathways is rudimentary and linking diseases in humans to biochemical pathways studied in cells is also difficult, gene network identification is a really hard problem and modeling a larger protein complex will be an important challenge.

The identification and validation of drug targets depends critically on knowledge of the biochemical pathways in which potential target molecules operate within cells. This requires a restructuring of the classical linear progression from gene identification, functional elucidation, target validation and screen development. One of the major goals of pharmaceutical bioinformatics is to develop computational tools for systematic *in silico* molecular target identification.

4 Concluding remarks

The advent of genomics offers means to expand the range of targets, the choice of potential drug targets thrown up by genomics data is overwhelming. One of the most important challenges for drug development, however, is to rapidly identify target proteins most appropriate to further development. Genomics and proteomics technologies have created a paradigm shift in the drug discovery process. Bioinformatics technology in the past decade has given birth to the new paradigm of a biology-driven process. There are many exciting developments to come in the field of target identification. Gene network technology creates cell and organ-level computer models able to simulate the clinical performance of drugs and drug candidates. By predicting how and why specific compounds impact human biology, gene networks technique may provide a glimpse of the signals and interactions within regulatory pathways of the cell. In fact, it is now possible to think of the whole pharmaceutical process as a computational approach, with confirmatory experiments at each decision-point.

There are several directions for future research. First, in the near future, data produced about cellular processes at molecular level will accumulate with an accelerating rate as a result of genomics studies. In this regard, it is essential to develop approaches for inferring gene networks from microarray data and other biological data effectively. The development of systematic approaches to finding genes for effective therapeutic intervention requires new models and powerful tools for understanding complex genetic networks. Secondly, owing to the reason that integrating the information from different types of networks may lead to the notion of functional networks and functional modules, to find these modules, we should consider the general question of the potential effect of individual genes on the global dynamical network behavior both from the view of random gene perturbation as well as intervention.

It should be emphasized that although computational tools and resources can be used to identify putative drug targets, validating targets is still a process that requires understanding the role of the gene or protein in the disease process and is heavily dependent on laboratory-based work. The new integrative technological developments in Systems biology [75], coupled with a number of 'omic' techniques, may lead to a breakthrough for the identification and validation of important drug targets in the future.

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