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Ranking effects of candidate drugs on biological process by integrating network analysis and Gene Ontology

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There are high preclinical attrition rates in current drug discovery. The efficient assessment approach in the high throughout candidate drugs screening still needs great improvement. We propose two hypotheses. First, both drug action process and biological process can be converted to a common space of gene or gene product profiling. Second, the strength of drug action on biological process can be realized in the context of biological network. Based on the above hypotheses, we establish an algorithm termed Network-based Assessment for Drug Action (NADA) to assess the action strength of candidate drugs on certain biological processes. Then NADA is used to prioritize the effects of six compounds from traditional Chinese medicine on endothelial cell migration, a simple process defined by Gene Ontology, in the biological network specific for a given pathological process, angiogenesis. The computational results are subsequently tested by the experiment on the migration of Human Umbilical Vein Endothelial Cells *in vitro*. The experimental ranks for six compounds generally agree with the predicted output of NADA. NADA also outperforms the DAVID and meet/min methods in terms of the experimental orders, suggesting that the network topological features may have a key role in catching the mechanistic relationship between drug action and biological process. Hopefully, the progress of network biology approaches for deciphering complex diseases will further expedite the preclinical screening and accelerate the development of treatment modalities.

network analysis, drug action, biological process, angiogenesis, traditional Chinese medicine

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Over the past decade, there has been a steady decline in the number of candidate drugs reaching the market. Strikingly, the decline is concurrent with the dominance of the assumption that the selective ligands binding to a single disease target is the most efficient [1]. Recent studies have revealed that the robustness of biological system can be better explained by the character of network [2]. Network analysis of disorders and disease genes linked by known associations indicates the common genetic origin of many diseases and supports the existence of distinct disease-related functional modules [3,4]. It is believed that essential human genes are likely to encode hub proteins, while the vast majority of disease genes tend to encode non-hub proteins, providing an intermediate habitat in terms of their biological importance and localizing in the functional periphery of the network [3,5]. Consequently, biological systems can recover against random deletion of any one node, but critically rely on hubs [6]. The understanding of biological networks underlying complex disease has led to the next paradigm in drug discovery, network pharmacology, characterized by changing from identification of "disease-causing" genes to perturbation in the disease-causing network [1,7]. Possible network attack strategies and methods to find target-sets for multi-target drugs were also proposed [8]. Thus, combining "omics" data with network biology may enable a new framework for developing a probabilistic approach to drug discovery [9].

Indeed, complex diseases indicate a breakdown of normal physiological system involving the intricate biological interaction and responding to environmental stimuli. As

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mechanistic understanding of disease requires more than integrating information on the expression and activities of disease-associated molecules, network analysis has been applied to many biological problems, providing a unique insight into understanding disease mechanism and a molecular basis for defining disease state [10,11]. In recent years, the relationships between approved drugs were addressed by combining available comprehensive drug information includeing genetic-disease associations, gene-expression and protein-protein interaction data [12]. Network analysis of integrated data sets further emphasizes the trend in pharmacology that drugs target the disease relevant genes [13]. Drug targets tend to be more interactive than average proteins but less interactive than essential proteins to a statistically significant degree. That the position of drug targets is between the essential hubs and redundant peripheral nodes in the biological networks suggests the possibility that statistical network analysis could be utilized to prioritize potential drug targets [13]. Moreover, not only drugs generally target on multiple targets but also drug targets are often involved with multiple diseases. Multi-target drugs complicate the relationship of pharmacologically relevant target molecules. The molecular mechanisms of diseases remain a major obstacle to the rationale selection of therapeutic targets. As the understanding gets deeper, drug target validation and identification become more and more complex [14].

These emerging findings in terms of network provide the motivation to the growing interest in drug discovery strategies utilizing known gene or gene product profiles associated with drugs rather than searching for single disease-causing genes and drug targets [15]. In the present work, we hypothesized that, firstly, drug action and biological process could be converted to a common space of gene or gene product profiling; secondly, the evaluation of the drug action on biological process could be realized through the interaction of two gene sets by considering network topological features in the context of disorder-specific biological network. Based on these assumptions, we established an algorithm termed Network-based Assessment for Drug Action (NADA) to assess the strength of drug action on certain biological processes. We chose candidate drugs from the commonly-used traditional Chinese medicine (TCM) full of potential drugs with uncertain function. Then, NADA was applied to prioritize the candidate drugs' effect on the endothelial cell (EC) migration, a key and relatively simple pathological process involving in a given disorder, angiogenesis. Angiogenesis is complex, with a number of molecular and cellular events that need to be spatial and temporal coordinated by a finely tuned balance [16]. Lastly, the computational predictions were experimentally validated and the robustness of NADA was demonstrated.

1 Materials and methods

1.1 Angiogenesis-specific biological network construction

The disorder-specific biological network needs to be constructed as background network of NADA (Figure 1). Angiogenesis, the growth of new blood vessels, is essential for organ growth and repair. An imbalance in this pathological process contributes to numerous malignant, inflammatory, ischaemic, infectious and immune diseases [17]. It is suggested that angiogenesis-specific biological network may help the rational design of angiogenesis-relevant drugs; whereas drugs with limited number of targets may fail to shift the robust angiogenic regulatory network [18]. Network construction is a critical procedure for understanding biological processes and the organizational principles of biological systems. In our previous work, we developed a combined literature mining and microarray analysis (LMMA) approach for building disorder-specific network [19]. Here, a part of the LMMA approach is used to construct the biological network of angiogenesis.

1.2 Candidate drugs and drug gene collection

Preparing candidate drugs interacting genes or gene products is required beforehand for NADA. We selected angiogenesis-relevant drug candidates from TCM, a precious resource for drug discovery and development [20]. The available genes/gene products (up/down regulated by drugs), termed as *drug genes*, were manually collected by reading literatures about the drug action from PubMed or China National Knowledge Infrastructure (http://www.cnki.net). The *drug genes* collected from multiple species were aligned to the human genome by orthologous mapping.

1.3 Gene collection of Gene Ontology biological process

On angiogenic activation, EC proliferate, degrade extracellular matrix, change their adhesive properties, migrate, avoid apoptosis, form tube-like structures, and eventually mature into new blood vessels. Therefore, the growth of vessels is a complex process involving a number of molecular and cellular events. Specific programs of gene expression are discovered to ensure an adequate angiogenic response [21]. In angiogenesis, EC migration is a simple biological process independent from other processes and its experimental validation can be taken easily. Thus NADA was applied to prioritize drugs for EC migration. Gene or gene products associated with EC migration (GO: 0043542), termed as *GO genes*, were collected from the Gene Ontology (GO) (http://www.geneontology.org/). The genes/gene products were presented in the PPI (protein-protein interaction) network from the HPRD database (release 7) [22].

1.4 Network parameters to assess vertex centrality

NADA is based on the measurement of the network topology of both set of *drug genes* and *GO genes*. To assess the

vertex centrality of these genes in the disorder-specific biological network, the following three parameters, namely Betweenness, Closeness and PageRank, were utilized.

$B(v) = \sum_{\substack{s \neq v \neq t \\ s \neq t}} \frac{n_{st}(v)}{n_{st}}$, B (Betweenness), is a centrality measure of the node in the network, where $n_{st}(v)$ is the number of the shortest paths going from node s to node t that pass through node v, and n_{st} is the number of the shortest paths going from s to t.

$C(v) = \frac{1}{\sum_{t \in V} d_{v,t}}$, C (Closeness), is the proximity degree of a node with all other network nodes, where V is the set of all other reachable nodes for v, and $d_{v,t}$ is the shortest path from node v to node t.

$P(A) = \frac{1-d}{N} + d\sum_{v \in L_v} \frac{P(v)}{N_v}$, P (PageRank) is determined by the immediate neighbors' centralities, and the neighbor with more importance contributes more than the less important one. In the formula for calculating PageRank value of node A, N_v denotes the number of links with node v, L_v denotes the set of nodes which have links to node A, N denotes the total number of all nodes in the network, and d denotes the damping factor from 0 to 1.

1.5 Ranking effects of drug candidates on EC migration

Six anti-angiogenic herbal compounds, namely aconitine, emodin, evodiamine, genistein, matrine and quercetin, were selected as drug candidates from TCM herbs [23]. Based on the curated *drug gene* data, we rank the effects of these six drug candidates on EC migration by using three methods, NADA, DAVID Functional Annotation Tool and the meet/min method. DAVID provides gene-GO term enrichment analysis to record the most relevant GO terms related to a given gene list [24]. The meet/min method is also used to rank drug effects by calculating the similarity between two gene sets without the network information [25], and is given by $C_{vw} = |N_v \cap N_w| / \min(|N_v|, |N_w|)$, where C_{vw} is the mutual coefficient between two sets, N_v is the interacting gene number of drug candidates (*drug genes*), and N_w is the gene number of a given GO term (*GO genes*).

1.6 Cell migration assay

The output of NADA in prioritizing anti-angiogenic candidate drugs was computed and experimentally validated on an angiogenic cell model, Human Umbilical Vein Endothelial Cells (HUVECs) migration assay. HUVECs were obtained from Cascade Biologics (Portland, USA) and cultured in duplicate according to procedures recommended by the manufacturer. Aconitine, emodin, evodiamine, genistein, matrine and quercetin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. The concentration of each drug designed according to related literature was 10 μM. HUVECs were allowed to grow into full confluence in 12-well plates precoated with 0.1% gelatin and then incubated with 10 μg/mL mitomycin C at 37°C, 5% CO_2 for 2 h to inactivate HUVECs. Monolayer inactivated HUVECs were wounded by scratching with 1 mL pipette tip. Fresh endothelial cell growth medium (ECGM) was added with or without different drugs. Images were taken by Nikon digital camera after 6 to 10 h of incubation at 37°C, 5% CO_2. The migration distance was quantified by the software provided by Olympus $[x]_0$ Inverted Microscope, and the inhibition percentage was expressed using untreated wells at 100% (*t*-test, $P<0.005$). At least three independent experiments were performed.

2 Results

2.1 Biological network for angiogenesis and drug candidate collection

By using the keyword of "Angiogenesis OR Neovascularization" (till February 9, 2007), we retrieved 49885 PubMed abstracts, from which 2707 genes with Entrez gene id were identified and served as nodes of angiogenesis-specific biological network. Then, two genes are considered to be linked if they have any PPI relations in HPRD or any pathway interactions in KEGG [19,22,26]. The interacting genes/gene products up or down regulated by each of the six anti-angiogenic drug candidates were manually collected from literature. The results were summarized in Table 1.

2.2 Sketch map of NADA

NADA takes the assumption that the drug action on the targeted biological process could be transferred to the relationship between drug-interacting gene sets and biological process involving gene sets in the context of network. The biological network specific for a given disorder is used to perform NADA and viewed as the background network. Then, we propose a Score of Topology (*ST*) in NADA to evaluate the relationship between both gene sets and rank

Table 1 Drug candidates and interacting genes/gene products

Drug candidate	Number of interacting genes/gene products	Top 3 curated drug-interacting genes/gene products
Aconitine	9	CHRNA7, MAPK1, CDK7
Emodin	46	PDGFB, AKT1, IFNA2
Evodiamine	20	TNF , RELA , NFKB1
Genistein	16	ESR1, ESR2, CFTR
Matrine	52	MYC,NRAS,TP53
Quercetin	37	ABCC1, ESR1, ABCC4

the drug actions on a targeted biological process. Here any gene-set-based biological process of interest can serve as the targeted process to perform NADA. For simplicity, we only consider the biological process with gene sets defined by GO to illustrate the usability of NADA.

As shown in Figure 1, *ST* is derived from the topological features of the background network. A map of protein-protein interactions illustrates that a few nodes with a very large number of links, which are often called hubs, hold these nodes together [27]. In protein interaction networks, highly connected nodes (hubs) avoid linking directly to each other and instead connect to proteins with only a few interactions. This effect reduces the probability of cross talk among various functional modules and enhances the network robustness by decentralizing effects of deleterious perturbations [28]. We infer that vertex centrality and the distance between nodes play critical roles in the disorder-specific network. So we assume that, the more important the drug-target gene or gene product (as node in the background network) is, the stronger effect the drug will produce; the more adjacent gene sets locate in the network, the stronger the action is. Thus, we define a topology-dependent score, *ST*, to evaluate not only the centrality, namely the *Vertex Centrality* (VC_D and VC_G for *drug gene* and *GO gene*, respectively), of a network node affected by the candidate drug, but also the network distance between *GO* (*EC migration*) *genes* and *drug genes*. Then *ST* is given by

$$ST = \frac{1}{2} \times \left(\frac{\sum_i VC_D(i) \times \exp\left(-\min(d_{i,j})\right)}{\sum_i VC_D(i)} + \frac{\sum_j VC_G(j) \times \exp\left(-\min(d_{j,i})\right)}{\sum_j VC_G(j)} \right),$$

where VC_D and VC_G are calculated by the three combined types of network topological parameters including Betweenness [2], Closeness [29] and a variant of Eigenvector, PageRank [30] through Principal Component Analysis (PCA); minus exponential function is utilized to weigh the interaction of two gene sets based on the shortest path length, and $\min(d_{i,j})$ is the minimum shortest path from node i of $drug_n$ to all the nodes of *GO* (*EC migration*) *genes* while $\min(d_{j,i})$ is from node j of *GO* (*EC migration*) *genes* to all the nodes of $drug_n$. Here we only consider the nearest connection between genes of $drug_n$ and of *GO* (*EC migration*) in the background network. The two terms in the brackets are dual and enable the measure of the drug action strength for a given biological process.

2.3 Computational and experimental ranks

Anti-angiogenic candidate drugs separated from TCM herbs, namely matrine, quercetin, emodin, evodiamine, genistein and aconitine, are ranked as the given drugs ($Drug_n$ in Figure 1) [31]. NADA ranks the drug candidates depending on the *ST* scores and the results are shown in Table 2. Among all drug candidates, matrine reached the highest in the action strength on *EC migration*. Consequently, we conducted an experimental validation for NADA predictions. The output of NADA and the results of cell migration assay are listed in Table 2 and illustrated in Figure 2. It was observed that six candidate drugs all inhibited *EC migration* and the inhibiting strength of matrine reached the highest during 9 h. The experimental rank order of the candidate drugs is mostly identical with those produced by NADA. The rank of candidate drugs was also achieved by DAVID Functional Annotation Tool [24] and meet/min method, but distinctly different with the experimental orders (Table 2).

2.4 Parameters and properties of NADA

NADA is a new edition of our NIMS (Network-Based Iden-

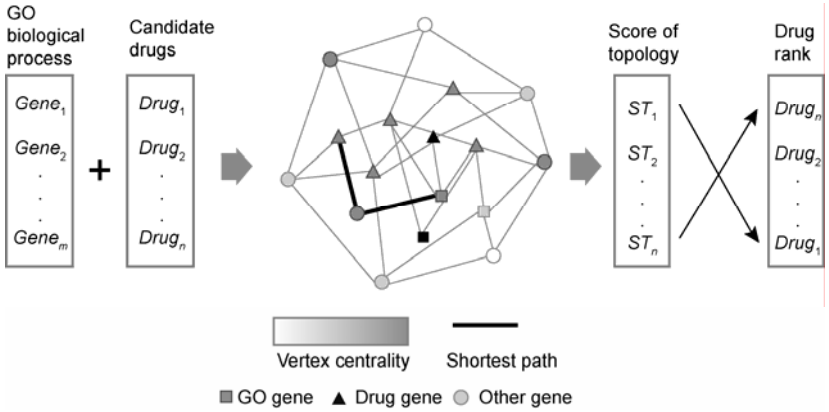

Figure 1 Sketch map of NADA. For a given GO biological process which has m involving genes or gene products ($Gene_1$, …, $Gene_m$) and n candidate drugs ($Drug_1$, …, $Drug_n$), all *drug genes* are collected and mapped to the biological network specific for a given disorder such as angiogenesis. Then, for each candidate drug ($Drug_x$), *ST* (Score of Topology) is obtained by calculating node importance for each *drug gene* and *GO gene*, as well as the shortest path between *drug gene set* and *GO gene set*. Then, *ST* is used to rank the drug action strength for n candidate drugs.

Table 2 Ranking effects of six compounds from traditional Chinese medicine on endothelial cell migration process by experiment, NADA, DAVID and meet/min methods respectively

Drug candidates	Experimental rank	NADA rank	DAVID rank	Meet/min rank
Matrine	1	1	2	5 [b)]
Quercetin	2	2	3	2 [b)]
Emodin	3	3	1	2 [b)]
Evodiamine	4	4	4	5 [b)]
Aconitine	6	5	5 [a)]	4
Genistein	5	6	5 [a)]	1

a) Aconitine and genistein are both ranked 5 by DAVID tool, for they both have no genes involved in *EC migration* term; b) Quercetin and emodin are both ranked 2 by the meet/min method, for they have the same number of interacting genes or gene products mapped in angiogenesis-specific biological network. So are matrine and evodiamine.

tification of Multicomponent Synergy) method for the prediction of synergistic drug combinations [32], while NADA is developed to evaluate drug action on the biological process. In NADA, three measures, namely Betweenness, Closeness and PageRank are integrated by PCA and employed to capture the network-based *Vertex Centrality* (VC_D) of genes/gene products associated with drug effect and the target biological process. In undirected angiogenesis network, we found that three measures were highly correlated and the majority (94.81%) of their variance can be explained by the primary eigenvector. We further compared the ranks of all drugs by using different combinations of the three centrality measures, and high SRCC (spearman rank correlation coefficient) was detected, indicating the robust performance of NADA in aspect of network topological parameters.

The robustness of NADA to both *drug genes* and the angiogenesis-specific biological network was also addressed referring to its previous edition, NIMS [32]. Firstly, 10% of the total *drug genes* were removed or added randomly each time and the added genes were randomly selected from the angiogenesis-specific network. Each addition or removal of genes was repeated 100 times. Interestingly, the SRCC was relatively stable in the adding gene procedure whereas decreased dramatically in the removal procedure, suggesting that the score of NADA may be mainly determined by some key genes of each drug, and the rank results will keep relatively stable as long as these key genes are reserved. Next, for the angiogenesis-specific network, we deleted or imported additional interactions at different percentage while maintaining the size of the network, each repeated 20 times, and measured the score respectively. By calculating the correlation with the original NADA score, we found that the SRCC was quite stable even 50% edges were added or removed, indicating that NADA is insensitive to both noise and incompleteness of the background network [32].

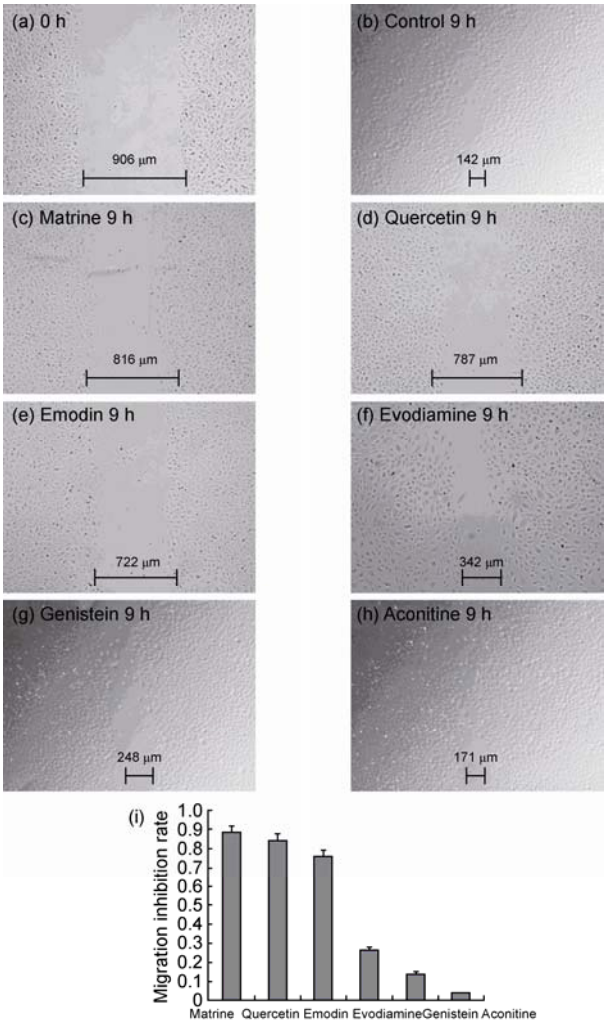

Figure 2 The anti-endothelial cell migration assay results. (a) The scratch made at 0 h; (b) Human Umbilical Vein Endothelial Cells (HUVECs) treated without drug migrated after 9 h; (c) HUVEC migration was most inhibited after treated with matrine for 9 h; (d) The HUVECs treated with quercetin for 9 h; (e) The HUVECs treated with emodin for 9 h; (f) HUVECs treated with evodiamine for 9 h; (g) HUVECs treated with genistein for 9 h; (h) HUVEC migration was the least inhibited after treated with aconitine for 9 h; (i) the migration inhibition rate of HUVEC migration for all six drug candidates.

3 Discussion

Understanding the relationship of human genes or gene products as well as the molecular basis for complex disease, drug efficacy and toxicity is a fundamental element in drug discovery. The advent of high-throughput technology has changed biomedical research into a data-rich discipline. Researchers have applied advanced analytical platforms, high throughput screenings, as well as quantitative structure-activity relationships-based virtual screenings to the assessment of drug actions and toxicity [33–35]. However, the poorly understood biocomplexity at various levels from the cell to the whole organism may frustrate the attempt to deeply interpret drug action mechanisms [36–38]. Utilizing

the large-scale biological data sets to obtain reasonable biological analysis about whole systems is a great challenge [39]. Systems biology promises to integrate multivariate biological information and measure multiple features of complex systems to better understand these problems. Advances in system biology, especially network biology suggest that simultaneous interventions at multiple nodes is required for modifying phenotypes in complex diseases [40]. The extensive generation of genomic, transcriptomic, proteomic, metabolomic and interactomic data provides the basis for achieving biological network [41], which further creates a new configuration for comprehending the molecular mechanism of drug-disease interactions [3,5,13,42]. In parallel with the exponential increasing data sets, many computational approaches evolve and become broadly used in drug discovery [43].

The potential of the gene expression profiling was underscored for unraveling the complex mechanisms of drugs actions and predicting drug action outcomes [44]. On the assumption that drug action on the targeted biological process could be converted to corresponding gene or gene product profiling and their interactions in the background network, we develop a computational approach, NADA, to prioritize the drug action strength on the given biological process. In NADA (Figure 1), we mainly make use of the network topological features formed by genes or gene products involved in drug action and biological process to define a topology-dependent score, *ST*, which evaluates not only the vertex centrality of a network node but also the network distance between both gene sets associated with drug candidates and biological process.

NADA integrates both the gene set information and the network topology information. In our case study for prioritizing six anti-angiogenic herbal compounds, NADA reported good performance and mostly agreed with the experimental ranks for drug candidates' effects on EC migration (Table 2, Figure 2). NADA also outperforms the DAVID and meet/min methods in terms of the experimental orders, suggesting that the network topological features which are employed by NADA but neglected by the other two methods, may have a key role in catching the mechanistic relationship between drug action and biological process. These results indicate that the information of drug-interacting gene or gene-product set reflects the character of drug, since a close relationship between the connectivity of nodes and drug action is obtained [45], and the therapeutic effect could be dramatically affected by topology of the perturbed nodes [46]. Furthermore, although our analysis was limited by the relatively scarce possibly inaccurate amount of public *drug gene* data and literature databases, as well as by the incomplete mapping of the background network, NADA was shown to be robust to collected *drug genes* if the key genes were reserved and to the background network although the available networks were still incomplete and biased [47]. It may be inferred from the fact that the differentiated expression genes and gene products of drug action were relatively downstream and located peripherally in the network, so their individual interference is hard to change the overall state.

Recently, the focus of drug discovery has shifted from targeting a single gene towards the systemically targeting network state, leading to the novel paradigm of "biological network, complex diseases". TCM with its abundant clinical practice may be a splendid example of system therapy and a rich resource for accelerating the shift of this paradigm. Some of the TCM herbal formula are found to potentially regulate the related biological network [48]. Here we demonstrate that the action of six TCM compounds on angiogenesis-related biological process can be explicated by the NADA approach. Among six compounds, matrine extracted from an anti-angiogenesis herbal formula *Qing-Luo-Yin* [49,50] has relatively stronger anti-EC migration action. We believe that the application of network biology approaches in TCM may facilitate the comprehension of the function of TCM herbs and herbal formulae, and then accelerate the modernization of TCM.

The realization that drugs against many non-normal states require miscellaneous activities to be efficacious may indeed inspire designing drugs that perturb biological networks rather than individual targets. Recent successes in the field of network biology have attracted the interest to see how systemic approaches might accelerate the drug discovery process. A deeper understanding of the cell networks and molecular basis that drive pathological processes will trigger novel therapeutic strategies, ultimately leading to the development of more efficacious and safer drugs. NADA is currently the pilot study on computationally prioritizing drug action in terms of biological process. The preliminary results demonstrate that NADA is efficient for identifying drug action on relatively simple biological process, serving as a promising beginning in elucidation of the inter-relationship between complex diseases and corresponding interventions.

NADA presently is limited to evaluate the drug action strength, but not drug action mode. For any candidate drugs, the drug action strength can be ranked, but whether the drug inhibits or promotes *EC migration* needs experimental clarification. Accordingly, more researches on NADA are still required to explain the association between drug action mode and the gene expression levels that are upregulated or downregulated, drug action strength and the regulated variations that are more or less, as well as the construction of more accurate background network. It is thus evident that NADA may benefit from more refinement and improvement by integrating multilayer, quantitative and large-scale "omics" information. Moreover, based on the rationale of NADA, we can further propose a novel concept of "network target", namely the gene products with typical topological / dynamic features in disorder-specific network and responsible for drug action or side-effect. The method of NADA will also be extended and developed to explain the features of "net-

work target" and thus aid pharmacology design in the future.

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