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A Network Target-based Approach for Evaluating Multicomponent Synergy

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Abstract Evaluation of multicomponent synergy is a critical point in current drug combination studies. However, it is still an ongoing challenge to prioritize the synergistic combination from various pharmacological agents in a high throughput manner. Here we proposed a network target-based approach termed NIMS (Network target-based Identification of Multicomponent Synergy), and showed that NIMS can not only recover the agent pairs with known synergistic effects, but also successfully predict synergistic agents from anti-angiogenic traditional Chinese medicine.

Keywords Network target; Multicomponent synergy; Traditional Chinese medicine; Angiogenesis

1 Introduction

The multicomponent therapeutics, in which two or more agents (chemical substances or herbs) interact with multiple targets simultaneously, is considered as a rational and efficient form of therapy designed to control complex diseases [1,2]. One of the fundamental advantages of multicomponent therapeutics is the production of "synergy", that is, the combinational effect to be greater than the sum of the individual effects, making multicomponent therapeutics a systematic approach, rather than the reductionism of an additive effect. Understanding multicomponent synergy is critical for developing a novel strategy to conquer complex diseases. It is believed that combinations of agents can effectively reduce side effects and improve adaptive resistance, thereby increasing the likelihood of conquering complex diseases, such as cancer, in a synergistic manner [3].

Evaluation of multicomponent synergy is usually implemented experimentally in a case-by-case approach [4]. Although large-scale experimental methods have been launched to screen favorable drug combinations by disease-relevant phenotypic assays [5], high-throughput identification of the synergistic agents arising from numerous individual agents remains an unresolved issue. Because the number of possible agent combinations is large, even in the case of a small collection of therapeutic agents, computational approaches that take advantage of the rapid accumulation of large-scale data may provide a more promising and desirable

method for multicomponent drug studies. Currently, computational methods for the evaluation of multicomponent therapeutics focus largely on two approaches. The first method is to identify and optimize multiple target interventions by modeling signaling pathways or specific processes and is usually applied to small scale problems [6-8]. One of limitations of this approach is the fact that cross-talk, or interaction among pathways, is widely present in complex diseases, suggesting that pathways should be integrated rather than treated separately [9,10]. The second approach is to measure the efficacy of drugs, especially multi-target drugs, by using network properties [11]. However, finding ways to evaluate multicomponent therapeutics and sort order for synergistic agent combinations is still a considerable challenge. Therefore, novel computational approaches are urgently required for feasible and efficient identification of multicomponent synergy.

In this work, we report a novel algorithm, called Network target-based Identification of Multicomponent Synergy (NIMS), to address the network target-based virtual screen and assess the synergistic strength of multicomponent therapeutics. Then, NIMS was used to prioritize synergistic combinations in TCM and a case study was subjected to experimental verification.

2 Results

2.1 Pipeline of NIMS

NIMS transfers the relationship among agents to the interactions among the target or responsive gene products of agents in the context of a network specific for a disease or pathological process. This hypothesis may be reasonable in many situations especially when synergy occurs only if the effects of individual agents are mediated through independent mechanisms. In NIMS, genes or gene products affected by individual agents are termed *agent genes*, and the disease-specific network serves as the network target of drugs.

Two elements of NIMS are Topology Score (*TS*) and Agent Score (*AS*) (**Figure 1**). *TS* is derived from topological features of the background network. Because the choke points or hub nodes may play a critical role in the network [12], the more important the agent-target gene or gene product in the background network is, the stronger effect that agent will produce. We also assume that if an agent pair produces a possible synergy, their target genes should be adjacent in the network. Thus, for a candidate agent pair, $agent_1$ and $agent_2$, we defined *TS* for $agent_1$ and $agent_2$ by given:

$$TS_{1,2}=\frac{1}{2}\times\left(\frac{\sum_i IP_1(i)\times\exp\left(-\min(d_{i,j})\right)}{\sum_i IP_1(i)}+\frac{\sum_j IP_2(j)\times\exp\left(-\min(d_{j,i})\right)}{\sum_j IP_2(j)}\right)$$

where $IP(v)$ is calculated by integrating three types of network topology parameters, Betweenness, Closeness and a variant of the Eigenvector PageRank [13], through Principal Component Analysis (PCA). The negative exponential function is utilized to weight the interaction of two agents based on the shortest path length. $\min(d_{i,j})$ is the minimum shortest path from node i of $agent_1$ to all the nodes of $agent_2$, whereas $\min(d_{j,i})$ is the minimum shortest path from node j of $agent_2$ to all the nodes of

agent$_1$. We only consider the nearest connection between *agent*$_1$ *gene* and *agent*$_2$ *gene* in the background network.

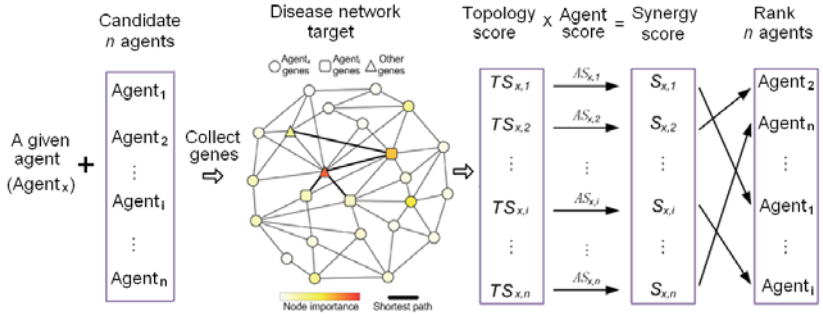

Figure 1. Pipeline of NIMS: ranking the synergistic effect of *n* agents paired with a given agent.

As agents with independent mechanism but treating similar diseases may be more likely to produce a synergistic effect, we introduced *AS*, a concept transferred from the phenotype similarity [14,15], to quantify the similarity score between two agent-target phenotypes and fine-tune the rank results. The agent-target phenotypes were identified from the OMIM database. If an *agent gene* falls into the gene set of a phenotype, this phenotype is considered as the corresponding agent-target phenotype. The similarity between two agent-target phenotypes quantifies the overlap of their OMIM descriptions and is calculated by a text mining method [14]. The *AS* for *agent*$_1$ and *agent*$_2$ is given by $AS_{1,2} = \frac{\sum_{i,j} P_{i,j}}{N}$, where $P_{i,j}$ is the phenotype similarity score for the *agent*$_1$-target *i*th phenotype and the *agent*$_2$-target *j*th phenotype, and *N* is the total number of phenotype pairs.

Ultimately, NIMS produces the synergy score, $S_{1,2}$, of *agent*$_1$ and *agent*$_2$ by calculating $S_{1,2} = TS_{1,2}$ x $AS_{1,2}$. High score means high synergy degree. To avoid the potential competition of both agents on the same targets, we only consider that the synergy score from 0 to 0.9 are the effective range.

2.2 Application and experimental verification of NIMS

We applied NIMS to prioritize synergistic agent pairs from 63 manually collected agents for treating a disease instanced by angiogenesis, a key pathological process in various diseases such as cancer and rheumatoid arthritis [16]. The NIMS synergy scores for all agent pairs ranged from 0.199270 to 0.012959. From the outputs of NIMS, we firstly checked the rank of five agent pairs with known synergy and found that they were ranked in the top layer. For example, the synergy scores of both 5-fluorouracil (5-FU) combined with vinblastine [17] (Rank = 2) and 5-FU combined with rapamycin [18] (Rank = 3) entered the top three.

Next, an anti-angiogenesis cell proliferation assay was conducted to validate NIMS predictions. A TCM anti-angiogenic alkaloid, Sinomenine [19], was selected as *Agent*$_x$ in **Figure 1**. Agent pairs were sampled from five intervals of the rank list

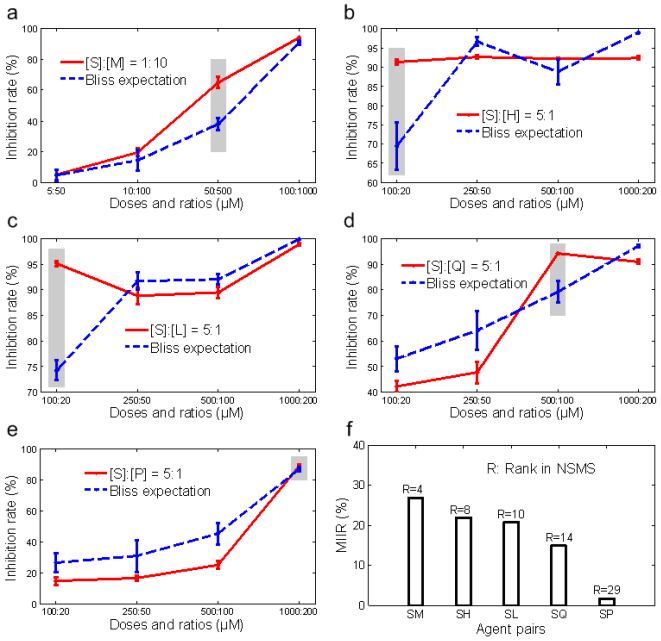

Figure 2. Anti-angiogenesis synergistic effects of five agent pairs. (a-e) The solid line denotes the inhibition rate of HUVEC cell proliferation in a dose-dependent manner. The dashed line denotes the additive effects calculated by the Bliss independence model. The gray column denotes the optimal dose and ratio of each pair. (f) The MIIR value for the synergistic effects produced by five agent pairs corresponds well with the NIMS ranks.

including all 62 agents matched with Sinomenine. Here, we only considered commercially available agents of known chemical structures. This restriction left five Sinomenine partners, namely Luteolin, Quercetin, Honokiol, Matrine and Paeoniflorin. To determine the synergy strength of the agent pairs, low-dose combinations with more than a 70% inhibition rate were regarded as effective [20]. Using the Maximum Increased Inhibition Rate (MIIR) measure for each combination (**Figure 2**), we found that the highest MIIR 26.83% was reached by Sinomenine combined with Matrine ((S):(M)), whereas the lowest MIIR 1.86% was reached by Sinomenine combined with Paeoniflorin ((S):(P)). This rank order of agent pairs is identical to the order predicted by NIMS.

2.3 Robustness of NIMS

NIMS integrated three measures, namely Betweenness, Closeness and PageRank to capture node importance, $IP(v)$ from different aspects. In the undirected angiogenesis network, we found that all three measures are highly correlated and the majority (94.81%) of their variance can be explained by the primary eigenvector. The robustness of NIMS was also addressed with respect to both *agent genes* and the background network. By adding or removing *agent genes* randomly, the permutation test results showed that the Spearman Rank Correlation Coefficient (SRCC) was

relatively stable when adding genes, but the SRCC decreased dramatically when some typical genes were removed (**Figure 3a-b**). This suggested that the NIMS synergy score may be determined largely by some key *agent genes*, and the rank results will remain relatively stable as long as these key genes are preserved. Such phenomena also agree well with that the power law networks are robust with respect to deletion of random nodes, but fragile with respect to deletion of hubs [21]. Moreover, by deleting or importing additional interactions at different percentages in the angiogenesis network, we found that the SRCC, with the original NIMS score, was quite stable even when 50% of the edges were removed or added (**Figure 3c**).

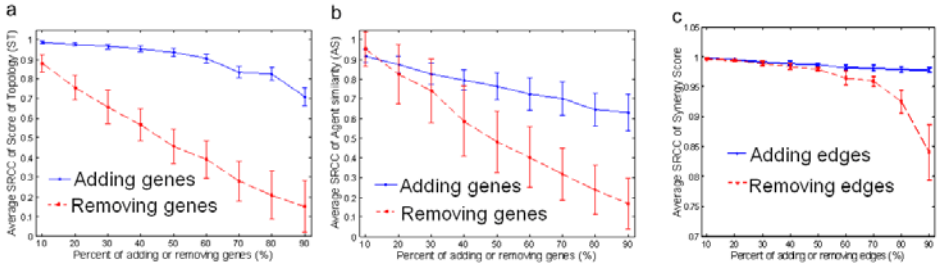

Figure 3. Permutation tests to assess the robust performance of NIMS. The permutation tests include (a) *TS*, (b) *AS*, and (c) the background network calculated by the average SRCC between the permutation outputs and the original scores.

2.4 Comparison with meet/min

To determine whether the synergy rank of agent pairs could be obtained from corresponding *agent genes* alone, regardless of network knowledge, we used the meet/min method, a similarity measurement between two gene sets that discards the network information [22], to rank the agent pairs. Compared with the experimental results, we found that the performance of the meet/min method was relatively poor in ranking the pairs containing Sinomenine.

3 Discussion

Recently, with the growing understanding of complex diseases, the focus of drug discovery has shifted from the well-accepted “one target, one drug” model designed toward a single target to a new “multi-target, multi-drug” model aimed at systemically modulating multiple targets [23]. In this work, we treated network as a target and established a novel approach, NIMS, to prioritizing the multicomponent synergy between agent pairs by combined network topology and agent similarity, with regard to agent target gene products as well as phenotypes. To demonstrate the capability of NIMS, we applied this algorithm to the prioritization of synergistic anti-angiogenesis agent pairs from an empirical multicomponent therapeutic system, TCM. Our results show that NIMS, especially when used against the angiogenesis network, could not only successfully recover known synergistic pairs, but also rank the anti-angiogenesis synergistic agents matched with a particular agent, Sinomenine (**Figure 2**). These findings demonstrate the effectiveness of NIMS as a tool for screening multicomponent synergy, which is also flexible to allow for connection

and collaboration with other segmental and global modeling methods [8,25].

NIMS uses both *agent gene* information and network topology information. We demonstrated that NIMS is robust to the collected *agent genes* if the key genes are reserved (**Figure 3a-b**). Moreover, NIMS is also relatively robust to the background network, although available networks, such as the PPI network, are still incomplete and biased (**Figure 3c**) [24]. We hypothesize that the following aspects of NIMS are responsible for such robust performance. Above all, the gene set information of each agent not only reflects the knowledge of agent similarity, but also determines the meet/min coefficient. We detected a relatively high correlation between the meet/min coefficient and the NIMS synergy score for all Sinomenine-related agent pairs, ensuring the stable performance of NIMS against different types of networks. Next, NIMS only uses a small fraction of the network around the nodes of *agent genes*. Thus, it is relatively insensitive to changes of the whole background network but very sensitive to changes in key genes.

For the limitations of NIMS, firstly, we only consider the responsive genes associated with a given disease or pathological process. It is believed that the more precise the disease-specific network target is chosen, the more accurate predictions will be obtained. Secondly, for *agent genes*, the present work only considered responsive genes rather than drug target associated with a limited number of TCM agents. Hopefully, with more rich and more precise information is revealed for more agents, these limitations could be alleviated and NIMS could be extended to more complicated conditions or more than two agents. Thirdly, as the initial effort for prioritizing synergistic agent combination in a computational framework, NIMS currently is a little bit simplified since it considers only part of the synergistic effects at the molecular level, and currently does not make the distinction for the synergistic and antagonistic effects. Further studies will be devoted to quantitative analysis of synergy, tissue-level synergy analysis, and pattern comparison between synergism and antagonism by integrating multilayer -omic data, spatio-temporal information as well as network state information such as the network Yin-Yang imbalance [26].

In summary, our work demonstrates that network target-based methods are of importance for estimating synergistic combinations, and NIMS can serve as a first-step approach for high-throughput identification of multicomponent synergy.

4 Methods

4.1 Data gathering

By reading more than 2,000 references regarding agent actions from both PubMed and the China National Knowledge Infrastructure (http://www.cnki.net), available *agent genes* and agent-target phenotypes were manually collected. A total of 736 non-redundant *agent genes* were obtained for 61 commonly used TCM agents (49 herbs and 12 herb-derived compounds) and 2 chemicals with known synergistic action. We also collected all the agent-target phenotype similarity scores from the study of van Driel *et al* [14] for calculating Agent Score (*AS*).

4.2 Angiogenesis network construction

The angiogenesis gene network was constructed by the LMMA method we developed previously [27] and used as the network target for NIMS. By using the keyword "Angiogenesis OR Neovascularization", we retrieved 49,885 PubMed abstracts (until Feb 9, 2007), in which 2,707 genes were identified with Entrez gene ids and served as nodes of the angiogenesis network. Two genes were considered linked if they had any relationship in the PPI from HPRD (release 7) or pathway interactions from KEGG.

4.3 NIMS robustness analysis

We conducted permutation tests and measured SRCC between the permutated and original *TS* or *AS* scores for the changes of three centrality parameters, collected *agent genes* as well as the background networks. In this step, *agent genes* were removed or added randomly from the angiogenesis network, changing 10% of the genes at a time. Each iteration of adding or removing genes was repeated 100 times. For angiogenesis network, we deleted or imported additional interactions at different percentages, each repeated 20 times, and measured the synergy score.

4.4 Angiogenesis *in vitro* assay

We employed the commonly-used Endothelial Cell Proliferation assay to evaluate NIMS predicted synergistic effects on angiogenesis. By using the Bliss independence model [28], the synergistic strength was determined by calculating: $MIIR=max(IR_{syn}-IR_{add})$, where IR_{syn} and IR_{add} denote inhibition rates of experimental measurement and the Bliss additive value of an agent pair at a certain dose/ratio.

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