INFERENCE OF PERSONALIZED DRUG TARGETS VIA NETWORK PROPAGATION

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We present a computational strategy to simulate drug treatment in a personalized setting. The method is based on integrating patient mutation and differential expression data with a protein-protein interaction network. We test the impact of in-silico deletions of different proteins on the flow of information in the network and use the results to infer potential drug targets. We apply our method to AML data from TCGA and validate the predicted drug targets using known targets. To benchmark our patient-specific approach, we compare the personalized setting predictions to those of the conventional setting. Our predicted drug targets are highly enriched with known targets from DrugBank and COSMIC ($p < 10^{-5}$), outperforming the non-personalized predictions. Finally, we focus on the largest AML patient subgroup (~30%) which is characterized by an FLT3 mutation, and utilize our prediction score to rank patient sensitivity to inhibition of each predicted target, reproducing previous findings of in-vitro experiments.

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1. Introduction

Precision medicine, an approach in which medical treatment is tailored for a specific group of patients, is an arising paradigm in medical research and practice. Indeed, it is well known that some drugs affect only a specific subgroup of patients, while even harming other patients suffering from the same disease [1-2]. In recent years, computational tools have emerged to stratify diseases into informative subtypes [3] and to predict drug sensitivity per subtype in order to optimally match patients with existing medical treatments [4].

In spite of these advances, the development of new treatments in the context of precision medicine is still scarce. Consequently, there is an increasing interest in computational prediction of drug targets. Previous works [6-9] used similarity among diseases to employ drugs designed for one disease to medicate another, as well as to prioritize new compounds as potential drugs. Lamb et al. [7] created a database containing ranked drug response gene expression profiles, allowing to query the database with a disease-specific genetic signature to identify drug response profiles that correlate with it. GBA [9] predicts novel associations between drugs and diseases by assuming that if two diseases are treated by the same drug, alternative drugs treating only one of them might treat also the other. Finally, Gottlieb et al. [6] predict novel associations between drugs and diseases by utilizing multiple drug–drug and disease–disease similarity measures for the prediction task. Some of the methods, such as [6-7] could be extended for personalized prediction of drugs, yet to this date efforts for personalized design of drugs had focused on experimental work [10] or small scale networks tailored for specific condition [11-12].

As drugs often act by inhibiting their targets, attempts were also made to predict candidates for drug targets by predicting the effect of gene knockouts. These attempts focused on metabolic drugs and used metabolic network models, testing the impact of in-silico deletion of genes on the network's fluxes. For example, Fatumo et.al. [13] simulated knockouts by deleting reactions from a metabolic network to identify enzymes essential for the malaria parasite Plasmodium falciparum. Papp et al. [14] used a metabolic flux model to predict the knockout fitness effect of nonessential genes in Saccharomyces cerevisiae. In their review of current paradigms for predicting inhibitory effects, Csermely et al. [15] conclude with the need for approaches allowing the examination of multi-targets inhibition, as our new approach allows.

In this work we present a novel approach to tackle the drug target inference problem from a personalized perspective using in-silico knockouts based on propagation methods in a protein-protein interaction (PPI) network. Figure 1 provides an overview of the method: we start from a general PPI network and personal disease-related data. We rely on the framework described by Vanunu et al. [16] to prioritize casual genes by network propagation. We perform multiple network propagations in order to simulate the current patient state, the patient state after gene knockouts (by removing the corresponding nodes from the network) and an estimated "healthy" state. We use these different states in order to rank the gene knockouts and retrieve a list of candidate drug targets.

The framework we present is general and could potentially be applied to any personalized disease-related data, with cancer being a pronounced candidate for application. Cancer is a wildly heterogeneous disease, in which a group of patient phenotypically categorized into the same cancer type (or even subtype) may have only little overlap in the underlying genotype. This is especially true in acute myeloid leukemia (AML), which has striking heterogeneity in gene mutations and expression aberrations across samples [17-19]. We therefore evaluate our performance by applying it to patients suffering from AML, based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/ of mutated and differentially expressed genes [19].

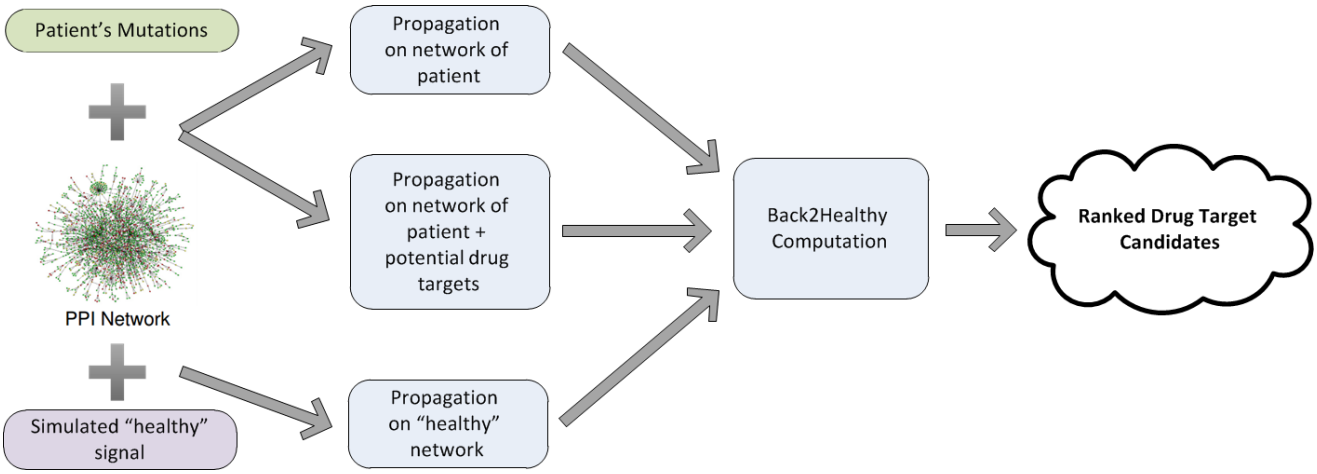

Fig. 1. An overview of the algorithmic pipeline.

2. Results

We present a novel approach to tackle the drug target inference problem from a personalized perspective using in-silico knockouts in a PPI network. As described in Figure 1, we start from a general PPI network and individual-specific disease-related data. We perform multiple network propagations in order to simulate the current patient state, the patient state after gene knockouts (by removing the gene's node from the network) and an estimated "healthy" state (see Methods). We use these different states in order to rank the gene knockouts and retrieve a list of potential drug targets.

To evaluate our performance we applied our method to TCGA gene expression and mutation data of patients suffering from acute myeloid leukemia (AML, see Methods for dataset description). First, we show that we can identify AML causal genes by synthesizing the individual propagations. Second, we show that by integrating results from a personalized knockout process we can infer potential drug targets and rank their efficacy in a patient or a subgroup of patients.

Our algorithm relies on network propagations to rank the relevance of different genes to a prior set. In order to set its parameters, we first tested the algorithm's performance in retrieving known causal genes for AML. The algorithm has two parameters (see Methods): α, determining the relative weight of the prior knowledge vs. the network in the scoring; and *P*, the prior set, according to which the propagation is carried out. We executed the algorithm using different settings for these parameters. To evaluate the results, we used three sets of known AML causal genes from KEGG and COSMIC, varying in confidence and size (see Methods). The application of the method to each patient resulted in a propagation score for each gene (excluding the prior set, to focus on novel discoveries). We aggregated the rank of each gene over all patients to yield a gene-based score, retaining the top 10% *affected* genes in the network. We then computed the hypergeometric enrichment of this set of genes with the different sets of known causal genes. All choices of α resulted in significant and similar p-values ($p < 10^{-5}$), which shows that the results are robust to the choice of α, as shown in Figure 2A. We use $\alpha = 0.9$ in the sequel. For the prior set, we tried four settings, defining P based on (i) mutated genes; (ii) differentially expressed genes; (iii) both, but running them separately and averaging the propagation scores obtained; and (iv) same as (iii) but taking the maximum scores rather than averages. Note that all types of mutations within coding genes were considered (missense, nonsense and silent). All prior knowledge variants resulted in significant p-values ($p < 10^{-5}$). The best variant was the first – setting P to be the set of mutated genes in each patient (Figure 2B), a choice which we use in the sequel. The mutated genes all belong to AML patients, but they are not limited only to AML-related genes.

The causal genes are thought to trigger malignant behavior by perturbing signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance [23]. In AML, cell survival and proliferation are enhanced through an aberrant signal pathway [24] represented in the KEGG database [21]. We computed the hypergeometric enrichment of the top 10% affected genes within the AML KEGG pathway (ID: hsa05221) and found that the affected genes comprise 15 out of 21 pathway components with a significant p-value ($p < 10^{-11}$), exceeding that achieved by using common mutated genes ($p < 10^{-7}$, mutations appearing in at least two patients) and capturing its downstream effect (Figure 3). It is interesting to note that although FLT3 is mutated in approximately 30% of the patients, it is not included in the top 10% affected genes after aggregation, underscoring the importance of a personalized approach.

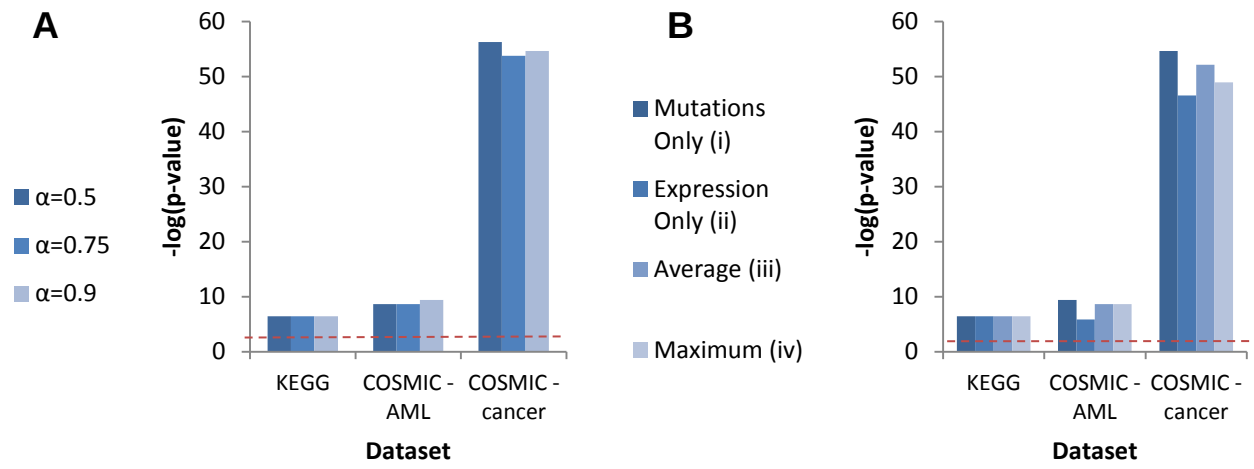

Fig. 2. Performance evaluation under different parameter (A) and prior knowledge set (B) choices. The red line denotes a p-value of 0.01.

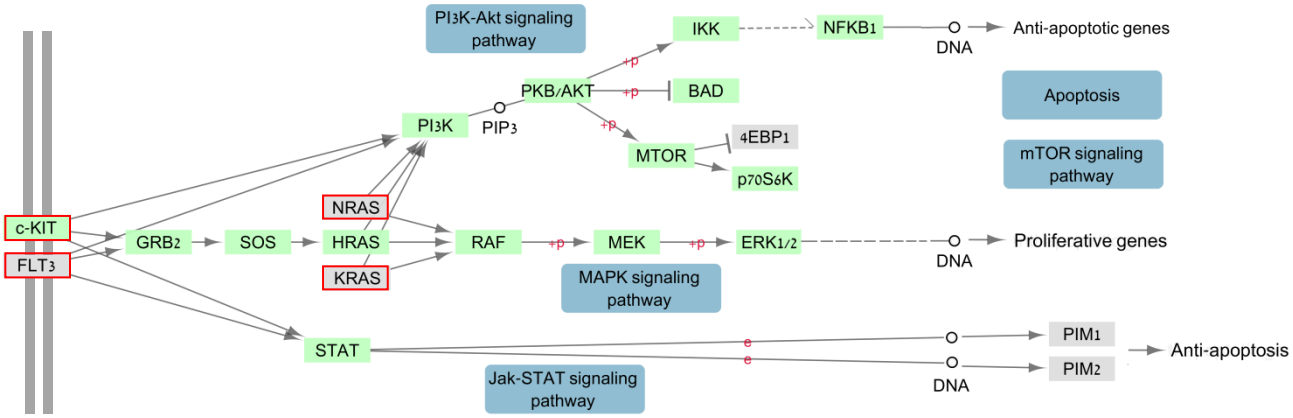

Fig. 3. The AML KEGG pathway, with top 10% affected genes (as predicted by our method) highlighted in green and commonly mutated genes framed in a red box.

The previous results imply that our propagation based scores are able to infer disease-related genes and agree with observations made by Rufallo et al. [25]. We hypothesized that good drug targets for the disease could be genes whose knockout is predicted to reverse the disease-related effects [7]. To identify such genes in-silico, we rerun the propagation based scoring while removing each gene in turn from the network, assessing the similarity between the obtained scores and those that characterize a "healthy" state. To this end, we use a Back2Healthy distance score (B2H; See Methods), taking the top scoring genes as our candidates for potential personalized drug targets. As above, we focus on non-trivial targets by excluding the patient's mutated genes from our ranking.

The process above infers drug targets for each patient individually. As information about personalized drug targets is very scarce and hard to validate, we aggregated the results over all patients, evaluating the results using known AML drug targets derived from the DrugBank database [26-29] and COSMIC [20]. The top 10% scoring genes were highly enriched with known drug targets from both sources (Figure 4A, DrugBank: $p < 10^{-5}$, COSMIC: $p < 10^{-10}$). In comparison, a naïve approach that focuses on common mutations (appearing in at least two patients), yields a set of candidate targets containing only one of the known targets ($p = 0.18$). To assess the personalized approach we took, we generated a "consensus patient", using common (appearing in at least five patients) mutated and differentially expressed genes, and applied our method to the "consensus patient". Applying the enrichment test described above, the results were insignificant (Figure 4B, DrugBank: $p = 0.22$, COSMIC: $p = 0.23$), underscoring the utility of a personalized approach.

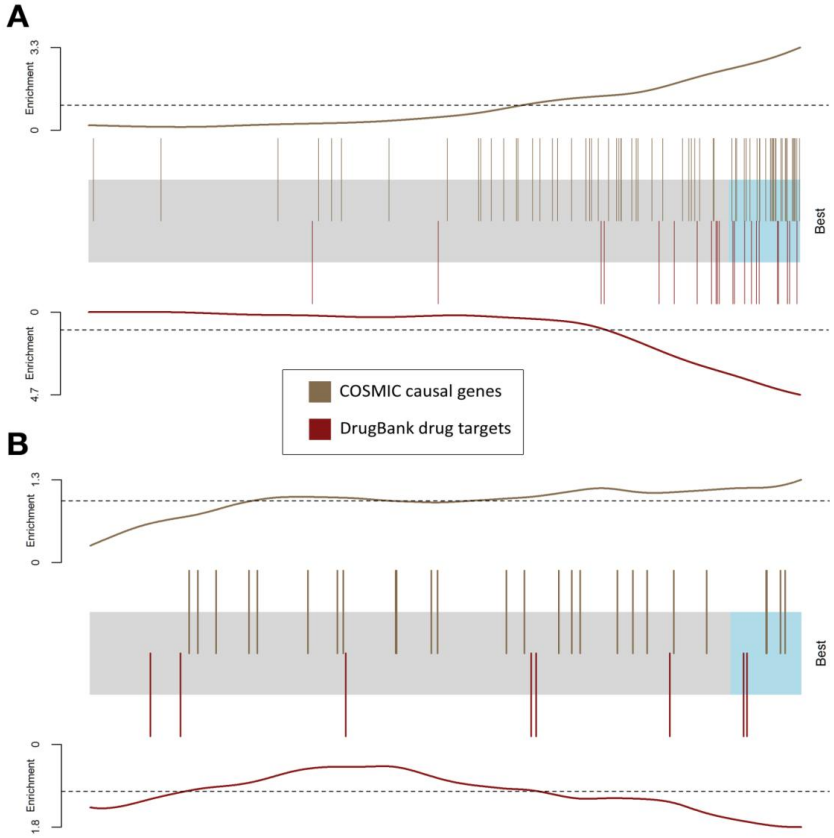

Fig. 4. Performance in drug target prediction. The candidate genes are represented by a shaded rectangle, where the top 10% are shaded cyan. Every overlaid bar stands for a single gene in a collection of known or potential drug targets. The bars are located according to their position in the candidate list generated by our method, where the rightmost bars represent the best candidates. Traces above/below the bar represent relative enrichment. (A) The barcode plot was generated by running our method on each AML patient independently and aggregating the results. (B) The barcode plot was generated by running a similar single pipeline on a “consensus” patient.

To further show the utility of our method, we used it to predict the sensitivity of the largest subgroup of AML patients – carriers of the FLT3 mutation – to known inhibitors. The following inhibitors were experimentally examined as potential drug targets and their influence on FLT3 mutated cell lines was carefully documented: Jin et al. [30] tested PI3K inhibitor and found FLT3 mutated cell lines to be poorly responsive to it; Nishioka et. al [31] showed that the MEK inhibitor caused those cell lines to respond moderately by leading to decreased abnormal proliferation, nearly resembling a healthy cell phenotype, yet showing unchanged abnormal levels of apoptosis; and Keeton et al. [32] demonstrated how PIM inhibitor caused FLT3 mutated cell lines to respond with high sensitivity, which led to development of the PIM inhibiting drug AZD1208. Our method shows in-silico sensitivity to PIM knockout, intermediate sensitivity to MEK knockout, and low sensitivity to PI3K knockout (Figure 5). These results corroborate the findings of [30-32].

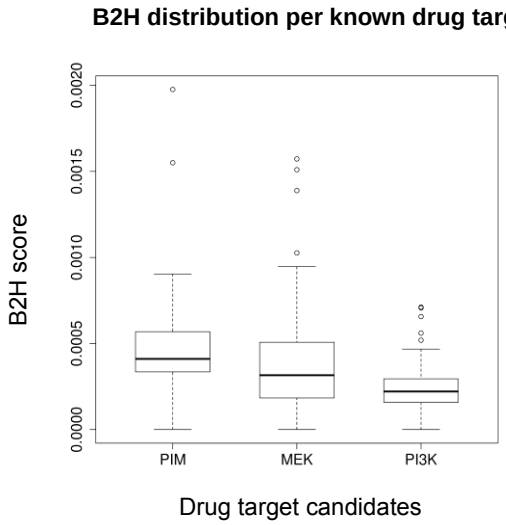

Fig 5. Sensitivity of FLT3 mutated cell lines, as predicted by B2H scores, corroborating the findings of [30-32] via in-vitro experiments.

3. Methods

3.1. *Computing propagation scores*

We use the network propagation method described in Vanunu et al. [16]. In the following we briefly describe it for the sake of completeness. The input consists of a network $G = (V, E, w)$ over a set V of proteins, where E represents the set of protein-protein interactions, and $w(u, v)$ represents the reliability of the interaction between u and v. In addition, a prior knowledge protein set P is given. The propagation process computes a scoring function $F: V \rightarrow \mathcal{R}$ that is both smooth over the network and accounts for the prior knowledge about each node.

To run the propagation process the weights are first normalized. Let W be the $|V| \times |V|$ matrix of initial weights, and let D be a diagonal matrix with $D[i,i] = \Sigma_j W[i,j]$. The normalized edge weight matrix is computed as $W' = D^{-1/2} W D^{-1/2}$. We further define a prior knowledge function $Y: V \rightarrow \{0,1\}$ such that:

$$\forall v \in V : \; Y(v) = \begin{cases} 1 & v \in P \\ 0 & v \notin P \end{cases}$$

We use the iterative procedure described by Law et al. [27] to compute F. Namely, starting with $F^{(0)} = Y$, we update F at iteration t as follows:

$$F^{(t)} = \alpha W' F^{(t-1)} + (1-\alpha) Y$$

The procedure is repeated iteratively until convergence, i.e., when:

$$\left\| F^{(t)} - F^{(t-1)} \right\|_2 < 10^{-4}$$

The final propagation score for each gene is its rank among all genes, where lower ranks mean higher $F(v)$ values. In case of ties, the ranks of the corresponding genes are averaged. The genes of the prior set are assigned the highest ranks to focus the algorithm on novel discoveries.

3.2. *The Back2Healthy distance score*

Let S_{before} , S_{after} be vectors of propagation scores for a chosen gene set (here, the set of differentially expressed genes of some patient) A, where S_{before} was generated by propagating on the original PPI network, while S_{after} was generated by propagating on a "knockout" network, where one of the genes was removed. We define the Back2Healthy (B2H) distance between S_{before} and S_{after} as follows:

Let k be the size of the prior gene set of the patient (the patient's set of mutated genes). For $1 \leq i \leq n$ $(n = 1000)$, we generate a score vector S_i for A by propagating the original PPI network and setting the prior knowledge set P to be k random nodes (disjoint from A) in order to simulate a "healthy" distribution of propagation scores for A.

Next, for $a \in A$, define

$$Q_{before_a} = \frac{\left|\{ 1 \leq i \leq n | S_i[a] < S_{before}[a] \}\right|}{n}$$

$$Q_{after_a} = \frac{\left|\{ 1 \leq i \leq n | S_i[a] < S_{after}[a] \}\right|}{n}$$

Hence, Q_{before_a} represents the quantile of $S_{before[a]}$ in our simulated distribution, and similarly for Q_{after_a}. Finally, $B2H(S_{before}, S_{after})$ is defined as:

$$B2H(S_{before}, S_{after}) = \frac{\Sigma_{a \in A} \left| Q_{before_a} - Q_{after_{la}} \right|}{|A|}$$

3.3. Data Sets

3.3.1. *Patient, network and drug target data*

The TCGA data portal [7] contains information on 200 clinically annotated adult cases of AML (updated to 29/04/2015). The data include whole-genome sequencing of the primary tumor and matched normal skin samples from 50 patients and exome capture and sequencing for another 150 paired samples of AML tumor and skin [19]. For 174 of the patients both mutation and expression data were collected. Genes exhibiting significant expression changes were determined by the COSMIC methodology [20], by computing their *z*-scores based on the sequencing platform.

To construct individual-specific networks, we projected the mutations and differentially expressed genes of an individual on a human PPI network taken from HIPPIE [33], which contains 186,217 interactions among 15,029 proteins. The projected networks have on average 7.6 mutated and 340 differentially expressed genes.

We retrieved the known targets of AML drugs from the DrugBank version 4.3 database [27], obtaining 22 drug targets overall.

3.3.2. *Known causal genes*

We use three sets of known AML causal genes, varying in confidence and size. 10 causal genes were collected from the KEGG database [21,22], 94 causal genes were taken from COSMIC (72 of which are in our PPI network), and a third set of 533 cancer causal genes were collected from COSMIC (363 are in the network).

4. Conclusions

The approach we presented succeeds in predicting known drug targets for AML and could potentially be applied to other diseases with mutation and expression information, such as other cancer types recorded in TCGA. It should be noted that our method is limited to mutations that affect proteins that are part of the PPI network. More careful consideration of mutations in non-coding regions could improve its sensitivity.

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