Computational and Structural Biotechnology Journal 21 (2023) 4446–4455

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Research article

A drug repurposing method based on inhibition effect on gene regulatory network

Xianbin Li [a,b], Minzhen Liao [a], Bing Wang [c], Xiangzhen Zan [a], Yanhao Huo [a], Yue Liu [a], Zhenshen Bao [a,b,*], Peng Xu [a,b,*], Wenbin Liu [a,**]

[a] Institute of Computational Science and Technology, Guangzhou University, Guangzhou, China
[b] School of Computer Science of Information Technology, Qiannan Normal University for Nationalities, Duyun, China
[c] School of Medicine, Southeast University, Nanjing, China

ARTICLE INFO

Keywords:
Drug repurposing
Inhibition effect
Gene regulatory network
Drug-disease association

ABSTRACT

Numerous computational drug repurposing methods have emerged as efficient alternatives to costly and time-consuming traditional drug discovery approaches. Some of these methods are based on the assumption that the candidate drug should have a reversal effect on disease-associated genes. However, such methods are not applicable in the case that there is limited overlap between disease-related genes and drug-perturbed genes. In this study, we proposed a novel **D**rug **R**epurposing method based on the **I**nhibition **E**ffect on gene regulatory network (**DRIE**) to identify potential drugs for cancer treatment. DRIE integrated gene expression profile and gene regulatory network to calculate inhibition score by using the shortest path in the disease-specific network. The results on eleven datasets indicated the superior performance of DRIE when compared to other state-of-the-art methods. Case studies showed that our method effectively discovered novel drug-disease associations. Our findings demonstrated that the top-ranked drug candidates had been already validated by CTD database. Additionally, it clearly identified potential agents for three cancers (colorectal, breast, and lung cancer), which was beneficial when annotating drug-disease relationships in the CTD. This study proposed a novel framework for drug repurposing, which would be helpful for drug discovery and development.

1. Introduction

In recent years, significant progress has been made in computing technologies, life sciences, and genomics [1]. However, drug discovery and development is not progressing as rapid as expected [2]. According to the previous records [3,4], a lot of new drugs failed in clinical trials. Meanwhile, it is estimated that it takes 10–15 years and 1 billion dollars to develop a new drug [5,6]. Hence, the journey of new drug discovery is not only time-consuming but also incurs substantial expenses and carries inherent risks. In order to overcome these challenges, drug repurposing has emerged as an effective strategy for discovering new indications for approved drugs, offering significant advantages to expedite the drug discovery process [7]. Recently, numerous computational-based drug repurposing approaches have been proposed to improve the efficiency of drug discovery [8–11]. These methods can generally be categorized into three main groups: signature-based [12], network-based [13], and pathway-based [14] methods.

Signature-based approaches primarily focused on identifying drug-disease pairs based on gene expression patterns. For instance, Lamb et al. [15,16] developed Connectivity Map (CMAP) database, which compared drug and disease expression profiles to discover potential drug candidates. Napolitano et al. [17] integrated chemical structures of drug, drug targets, and drug-induced gene expression for drug repurposing. Chen et al. [18] presented a drug repurposing method by using tissue/species-specific transcriptome data and drug-perturbed gene expression information from CMAP. Dudley et al. [19] identified potential drug-disease relationships by systematically comparing the gene expression characteristics of disease with that of drug compounds from CMAP. Drugs and diseases could share common genes on which drugs execute their functions. The more common genes, the stronger relationship between the drug and disease [20]. Several studies have been presented to discover drug-disease pairs based on their related gene expression information [21,22]. Meanwhile, many approaches have been developed according to the protein complexes shared by the drug

* Corresponding authors at: Institute of Computational Science and Technology, Guangzhou University, Guangzhou, China.
** Corresponding author.
E-mail addresses: bzsbao@163.com (Z. Bao), gdxupeng@gzhu.edu.cn (P. Xu), wbliu6910@gzhu.edu.cn (W. Liu).

https://doi.org/10.1016/j.csbj.2023.09.007
Received 22 March 2023; Received in revised form 5 September 2023; Accepted 7 September 2023
Available online 9 September 2023

and disease [23] and their common perturbed genes [24]. However, the signature-based approaches cannot be utilized to identify the drug-disease relationships without common genes.

There are other network-based methods than those using cross-network message diffusion, e.g. methods based on shortest paths. For instance, Zhang et al. [25] adopted a method based on network topological similarity-based inference to identify unknown drug-disease pairs. Liu et al. [26] applied random walk with restart in heterogeneous network to find new uses for FDA approved drugs. Zhao et al. [27] predicted drug-disease interactions by using graph representation learning method on heterogeneous information network. Furthermore, Cheng et al. [28] used an already available approach to identify candidate drugs for 220 million patients from Guney et al. [29] and it was based on network proximity. However, these methods did not consider drug-induced gene expression profiles.

Pathway-based approaches mainly identify drug–pathway relationships, where drugs perturbed gene expression within specific pathways, consequently affecting the pathway's function. Recent studies reported that pathway-based drug repurposing method was an effective strategy to discover potential candidates for thoracic aneurysms [30]. Li et al. [31] established a drug-target-pathway-gene-disease network to find new drug-disease pairs. Napolitano et al. [32] proposed a computational method for drug repurposing by integrating drug-induced gene expression and therapeutic target genes in given pathways. Yu et al. [33–36] presented computational approaches to discover potential drug-disease relationships based on different network models, such as module distance, random walk, triangularly balanced structure, and tissue-specific network [36]. Additionally, numerous studies have reported a close association between local region of the pathway and certain diseases [37–39]. These studies have successfully identified potential drugs whose targets are enriched within these local regions. For example, Li et al. [37] found that subpathways were closely associated with cancer occurrence and development. Han et al. [38] proposed an effective method when searching for subtype-specific drugs at the subpathway level. Nam et al. [39] developed a subpathway-based polypharmacology drug repurposing approach. However, they treated pathways or subpathways simply as gene sets and ignored their network structure information to discover potential candidate agents.

In this paper, we proposed a novel drug repurposing method called DRIE, which integrated gene expression profile and gene regulatory network to compute inhibition score using the shortest distance in the disease-specific network. The results demonstrated our method yielded superior performance over state-of-the-art approaches on eleven datasets. Furthermore, case studies showed that DRIE helped to find new drug-disease relationships that do not exist in Comparative Toxicogenomics Databases (CTD) [40], which was a literature-based resource containing gene-to-disease, drug-to-gene, and drug-to-disease associations. Our findings demonstrated that the top-ranked drug candidates have been already validated by CTD. In this regard, utilizing gene regulatory network provided us an alternative view to address the issue of low overlap gene between disease and drug, which was neglected by other methods. Additionally, it can clearly identify potential agents for three cancers (colorectal, breast, and lung cancer), which is beneficial when annotating drug-disease relationships in the CTD. In conclusion, we confirmed that our study opens up a new avenue for drug repurposing with new insights gained from the gene regulatory network.

2. Materials and Methods

2.1. Gene expression profile

We downloaded nine datasets from the Gene Expression Omnibus (GEO) database. These datasets contained colorectal cancer (GSE8671, GSE9348, and GSE23878), breast cancer (GSE31448, GSE42568, and GSE29044), lung cancer (GSE18842, GSE19188, and GSE19804), rheumatoid arthritis (GSE55235), and Alzheimer's disease (GSE5281). Gene expression profiles were generated with Affymetrix Human Genome U133 plus 2.0 array. These datasets were shown in Table 1.

Table 1
The gene expression datasets.

Dataset	GEO	Case/control	Ref
Colorectal Cancer	GSE8671	32/32	[41]
	GSE9348	70/12	[42]
	GSE23878	19/19	[43]
Breast Cancer	GSE31448	29/4	[44]
	GSE42568	104/17	[45]
	GSE29044	73/36	[46]
Lung Cancer	GSE18842	46/45	[47]
	GSE19188	91/65	[48]
	GSE19804	60/60	[49]
Rheumatoid Arthritis	GSE55235	10/10	[50]
Alzheimer's Disease	GSE5281	12/9	[51]

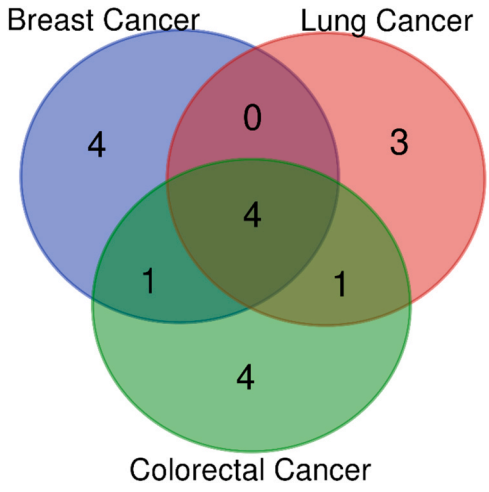

Fig. 1. Venn diagram of three cancer-related pathways.

2.2. Drug-perturbed gene expression profile

We downloaded drug-exposure gene expression profiles from the CMAP database [15], which consisted of 6100 instances and covered 1309 drugs. These instances were measured on five human cancer cell lines, which contained the breast cancer epithelial cell lines (MCF7, ssMCF7), the prostate cancer epithelial cell line (PC3), the nonepithelial leukemia cell line (HL60), and melanoma cell line (SKMEL5). Due to the little number of instances in ssMCF7 and SKMEL5, we only used three cell lines (MCF7, PC3, and HL60) for subsequent analysis.

2.3. Differentially expressed genes

We defined differentially expressed genes (DEGs) between tumor and normal samples as disease-related genes by 'limma' package [52]. In the CMAP database, we screened DEGs as drug-induced genes by comparing treated samples with the corresponding control samples (Fig. 2). The statistical significance threshold for our analysis was defined as p-value < 0.05. Notably, we did not establish a specific logFC (logarithm of fold change) cutoff in this study.

2.4. The construction of the disease-specific network

We downloaded 137 signaling pathways from the Kyoto Encyclopedia of Gene Genomics (KEGG). A signaling pathway was modeled by a graph in which nodes represented genes, and edges represented interactions between them, such as activation, inhibition, etc. There were

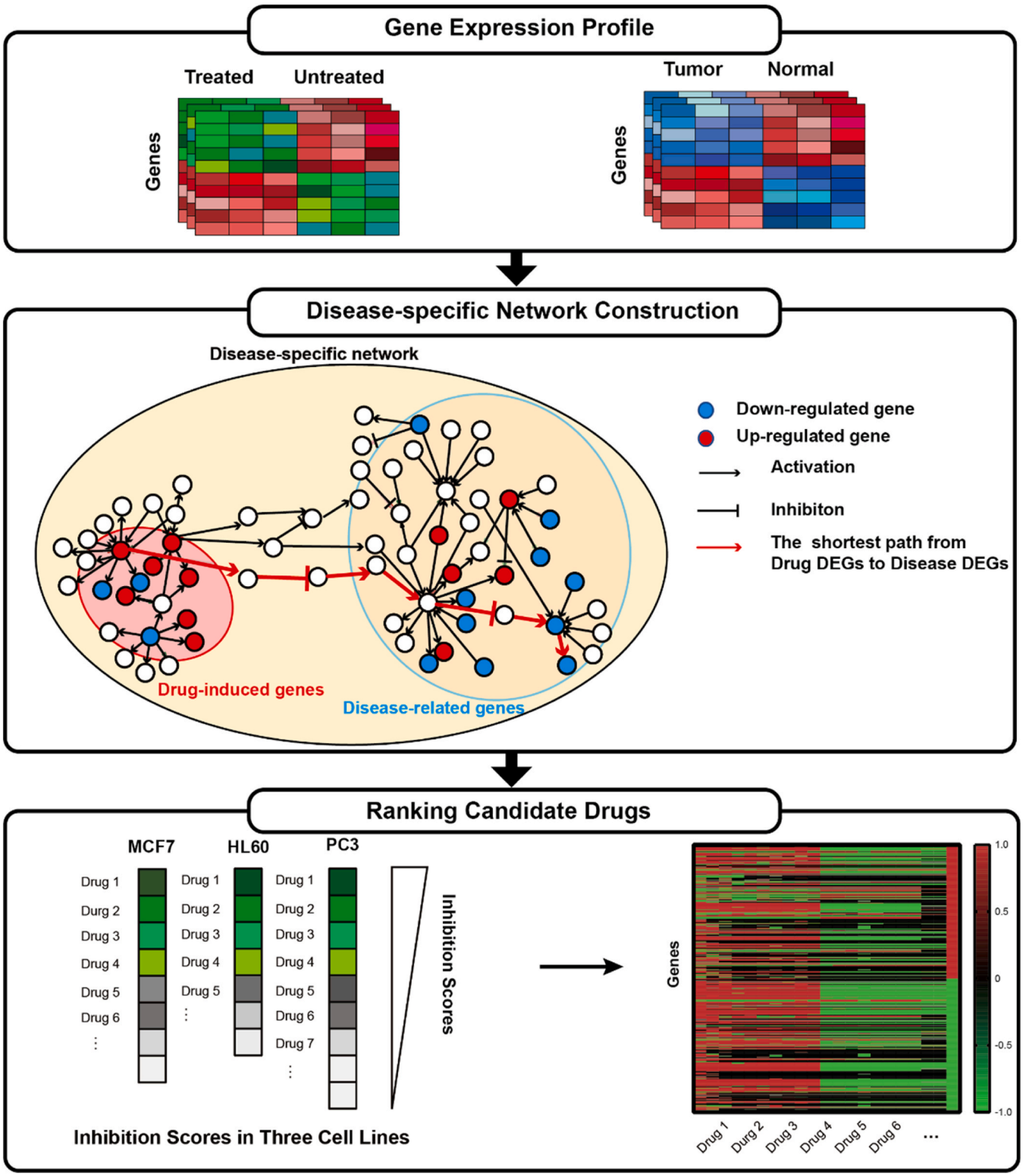

Fig. 2. The workflow of the DRIE method to predict candidate drugs for cancer treatment.

10, 9, and 8 pathways associated with colorectal, breast, and lung cancer in the KEGG database (Fig. 1). We established three disease-specific networks for colorectal, breast, and lung cancer based on these signaling pathways, respectively. In addition, rheumatoid arthritis and Alzheimer's disease contain 7 and 10 pathways, respectively.

We established a disease-specific network by conducting the union of all nodes and edges of disease-related signaling pathways. We applied the 'DrugDiseaseNet' package [24] to integrate disease-related signaling pathways that were represented by the adjacency matrices and derived a unified adjacency matrix.

Then, given disease-related pathways, disease-associated genes, and drug-perturbed genes, we computed the shortest paths connecting these genes in a disease-specific network. It meant that a drug-perturbed gene can be a source and a disease-related gene can be the destination of the

shortest path extracted from the disease-specific network (Fig. 2). The graph named disease-specific network represented all the interactions between drug-perturbed genes and disease-related genes, through all the interactions described in disease-related signaling pathways.

2.5. Calculating the inhibition score

We hypothesized that if the perturbation caused by a drug in the system was opposite to that of a disease, the drug may have the potential to treat the disease. Thus, we calculated inhibition scores for all drug-disease pairs in the disease-specific network, as shown in Fig. 2. First, we obtained the coefficient β_{ij} that represented the accumulation of the interaction type between drug-perturbed genes and disease-related genes.

$$\beta_{ij} = \prod_{k=1}^{L_{ij}} \beta_k \tag{1}$$

Where$\beta_k$represented interaction types among genes, including 1 (activation) and − 1 (inhibition). L_{ij} represented the shortest distance from gene i to gene j. A drug affected disease-related gene$j$, effect score E as follow:

$$E(j) = sign(\sum_{i=1}^{m} \frac{\beta_{ij} * \log_2(F_i)}{L_{ij}+1}) \tag{2}$$

Where m represented the number of drug-perturbed genes. F_i represented the fold change of drug-perturbed gene i. Then, an inhibition score for each drug,S, was calculated by using the impact from drug-perturbed genes to disease-related genes through the shortest path in the tissue-specific network, as follows:

$$S = \sum_{j=1}^{n} -E(j) * sign(\log_2(F_j)) \tag{3}$$

Where n represented the number of disease-related genes. F_j represented fold change of disease-related gene$j$.

Finally, we obtained the inhibition score for a drug-disease association by calculating the sum between drug-perturbed genes and disease-related genes n (Formula 3).

3. Performance evaluation

Drug-disease relationships were retrieved from Comparative Toxicogenomics Database (CTD, [40]). CTD was a database that provided curated data describing cross-species chemical-gene/protein interactions and gene-disease associations. Here, if there were associations between drug-disease in CTD database, we defined these drug-disease pairs as positive samples. The drug-disease associations not exist in CTD were regarded as negative samples.

To guarantee a reliable performance evaluation, we devised CMAP, Hyper, and Network proximity as benchmarks. We evaluated the efficacy of the methods in the aspect of AUPR, AUROC, Recall, ACC, and F1.

$$recall = \frac{TP}{TP+FN} \tag{4}$$

$$ACC = \frac{TP+TN}{TP+TN+FP+FN} \tag{5}$$

$$F1 = \frac{2TP}{2TP+FP+FN} \tag{6}$$

Where TP, FP, TN and FN denoted the numbers of true positive, false positive, true negative and false negative associations, respectively. AUROC was the area under the receiver operating characteristic (ROC) curve, which can be plotted by true positive rate and false positive rate.

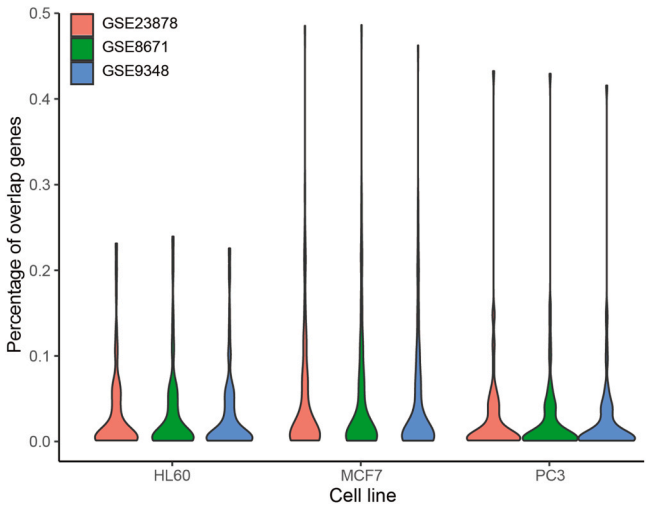

Fig. 3. The percentage of overlap genes between CRC-related DEGs (GSE8671, GSE9348, GSE23878) and drugs-perturbed genes in three cell lines.

AUPR was the area under the precision-recall curve, which can be plotted by precision and recall.

4. Baseline methods

To evaluate the performance of our proposed method, we compared DRIE with three state-of-the-art drug repurposing approaches listed below:

CMAP: Identify drugs that reverse cancer-associated gene expression signatures using Kolmogorov-Smirnov (KS) statistical method [53] between drug-perturbed genes and disease-related genes (ignore pathway and network) [15].

Hyper: It ranks drugs by using a hypergeometric test between drug-perturbed genes and disease-related genes [54].

Network proximity: It screens drugs by calculating the average shortest distance between drug-perturbed genes and disease-related

Table 2
Performance comparison in three cell lines based on nine datasets.

Cell line	Dataset	AUPR	AUROC	Recall	ACC	F1
MCF7	GSE8671	**0.752**	**0.669**	**0.638**	**0.650**	**0.695**
	GSE9348	**0.748**	**0.665**	**0.659**	**0.660**	**0.715**
	GSE23878	**0.807**	**0.702**	**0.729**	**0.690**	**0.750**
	GSE29044	**0.724**	**0.622**	**0.804**	**0.660**	**0.755**
	GSE31448	**0.680**	**0.642**	**0.585**	**0.630**	**0.635**
	GSE42568	**0.673**	**0.649**	**0.607**	**0.640**	**0.655**
	GSE18842	**0.823**	**0.714**	**0.642**	**0.660**	**0.710**
	GSE19188	**0.850**	**0.727**	**0.676**	**0.680**	**0.735**
	GSE19804	**0.849**	**0.732**	**0.670**	**0.660**	**0.720**
HL60	GSE8671	0.627	0.585	0.512	0.580	0.570
	GSE9348	0.667	0.616	0.523	0.590	0.585
	GSE23878	0.623	0.589	0.512	0.580	0.570
	GSE29044	0.654	0.594	0.423	0.526	0.500
	GSE31448	0.651	0.620	0.556	0.580	0.595
	GSE42568	0.606	0.595	0.568	0.535	0.600
	GSE18842	0.542	0.561	0.448	0.553	0.510
	GSE19188	0.539	0.561	0.538	0.586	0.575
	GSE19804	0.564	0.559	0.481	0.573	0.540
PC3	GSE8671	0.677	0.589	0.348	0.553	0.472
	GSE9348	0.709	0.603	0.363	0.533	0.477
	GSE23878	0.685	0.601	0.329	0.540	0.456
	GSE29044	0.553	0.554	0.302	0.500	0.380
	GSE31448	0.542	0.557	0.338	0.560	0.431
	GSE42568	0.582	0.575	0.337	0.533	0.435
	GSE18842	0.642	0.572	0.386	0.533	0.492
	GSE19188	0.680	0.591	0.390	0.553	0.500
	GSE19804	0.690	0.590	0.400	0.540	0.510

ACC: accuracy

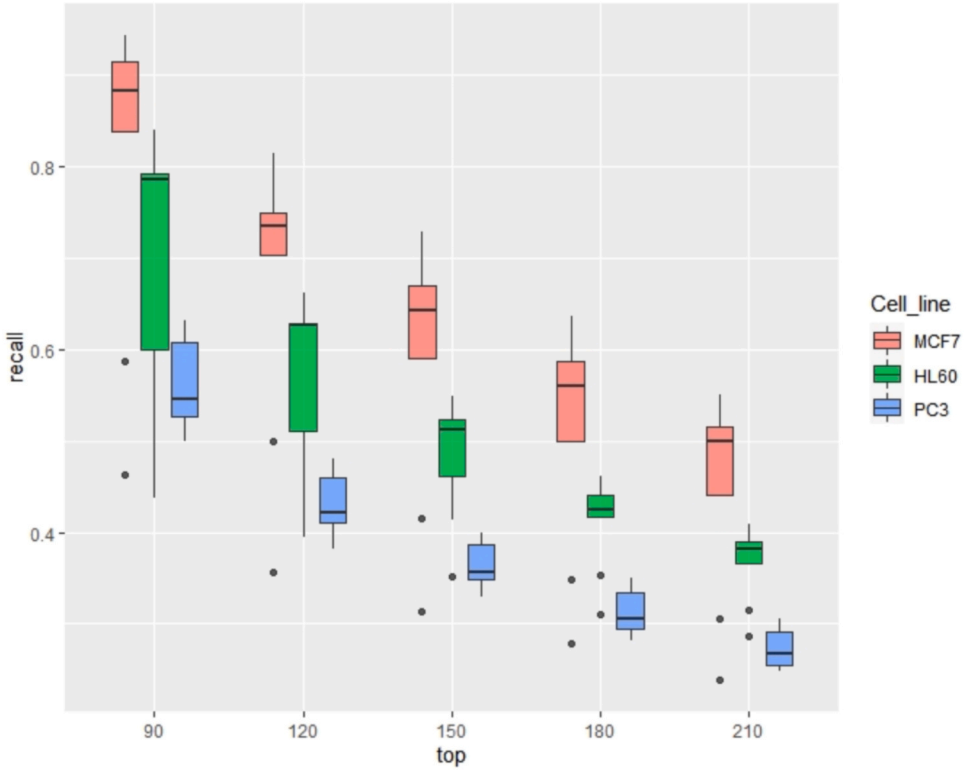

Fig. 4. Recalls of three cell lines at different top *k* cutoffs.

genes in the PPI network [28].

5. Results and Discussion

5.1. Overlap between drug-perturbed genes and disease-related genes

To analyze the overlap between drug-perturbed genes and disease-related DEGs, we applied these DEGs to validate common genes in three cell lines. Fig. 3 showed that the percentage of overlap genes was mostly less than 1% between colorectal cancer (CRC)-related DEGs (GSE8671, GSE9348, and GSE23878) and drugs-perturbed genes in three cell lines, and the overlap was largely consistent across the three cell lines (HL60, MCF7, and PC3). The results showed that the overlap percentage was very low. In addition, the overlap genes were also little in breast and lung cancer (Supplementary Figs. S1 and S2). Thus, it was intractable to directly evaluate the effect that drugs have an influence on disease. To solve the issue, we utilized gene regulatory network to measure the impact of drug on disease.

5.2. Performance evaluation in different cell lines

To validate the performance of our approach in different cell lines, we applied nine cancer datasets to identify top-rank drugs in three cell lines (MCF7, HL60, and PC3). According to Table 2, the results indicated that the average recall of DRIE in MCF7 were 67%, 64%, and 67% in colorectal, breast, and lung cancer, respectively. Our method in MCF7 significantly outperformed HL60 (58%, 55%, and 57%) and PC3 (54%, 53%, and 54%) cell lines. We obtained the recall for other cutoffs based on different datasets (Fig. 4). The results showed that the performance of MCF7 was the best in three cell lines. Thus, DRIE achieved the best performance for recall on the MCF7 cell line. Therefore, the following analysis were based on MCF7 cell lines to screen drug candidates.

5.3. Inhibition score from drug-perturbed genes to disease-related genes

To investigate the inhibition impact between drug-responsive genes and disease-related genes, we applied inhibition score to measure the strength of the relationship between drug and disease based on a disease-specific network. The drug with high inhibition score was more likely to be a potential treatment for CRC. Fig. 5 showed the heat map of the correlation between the top 10 drug perturbation genes and disease-related genes in the MCF7 cell line on three CRC-related datasets. The results showed that 65% metformin-perturbed genes upregulate the down-regulated genes of disease, and 22% metformin-perturbed genes downregulate the up-regulated genes of disease, but 50% metformin-perturbed genes upregulate the up-regulated genes. The results indicated that most drug-perturbed genes of the top 10 drugs are negatively correlated with disease-related genes in three datasets.

To verify the robustness of the model, we utilized the DRIE approach to predict the top 10 drugs and compare the results in three datasets. To provide a general comparison, the top 10 drugs for each ranked drug list in the GSE8671, GSE9348, and GSE23878 datasets were leveraged. As shown in Fig. 6, 50% (5/10) of drugs all exist in the three datasets, which demonstrated the great robustness and stability of our method in the different datasets. For example, there were five drugs, including trichostation.A, anisomycin, metformin, etoposide, and pyrvinium simultaneously exist in the top 10 drugs of GSE23878, GSE8671, and GSE9348 datasets.

5.4. Case study

To test the ability to predict novel drug-disease associations, we utilized our method to identify potential drugs in datasets of three well-known malignant cancers: colorectal, breast, and lung cancer. There were 330, 511, and 537 drugs were associated with colorectal, breast, and lung cancer in CTD database, respectively. We obtained the top 10 DRIE-predicted drugs for potentially treating the three cancers based on the MCF7 cell line (Supplementary Table S1, S2, S3), respectively. The

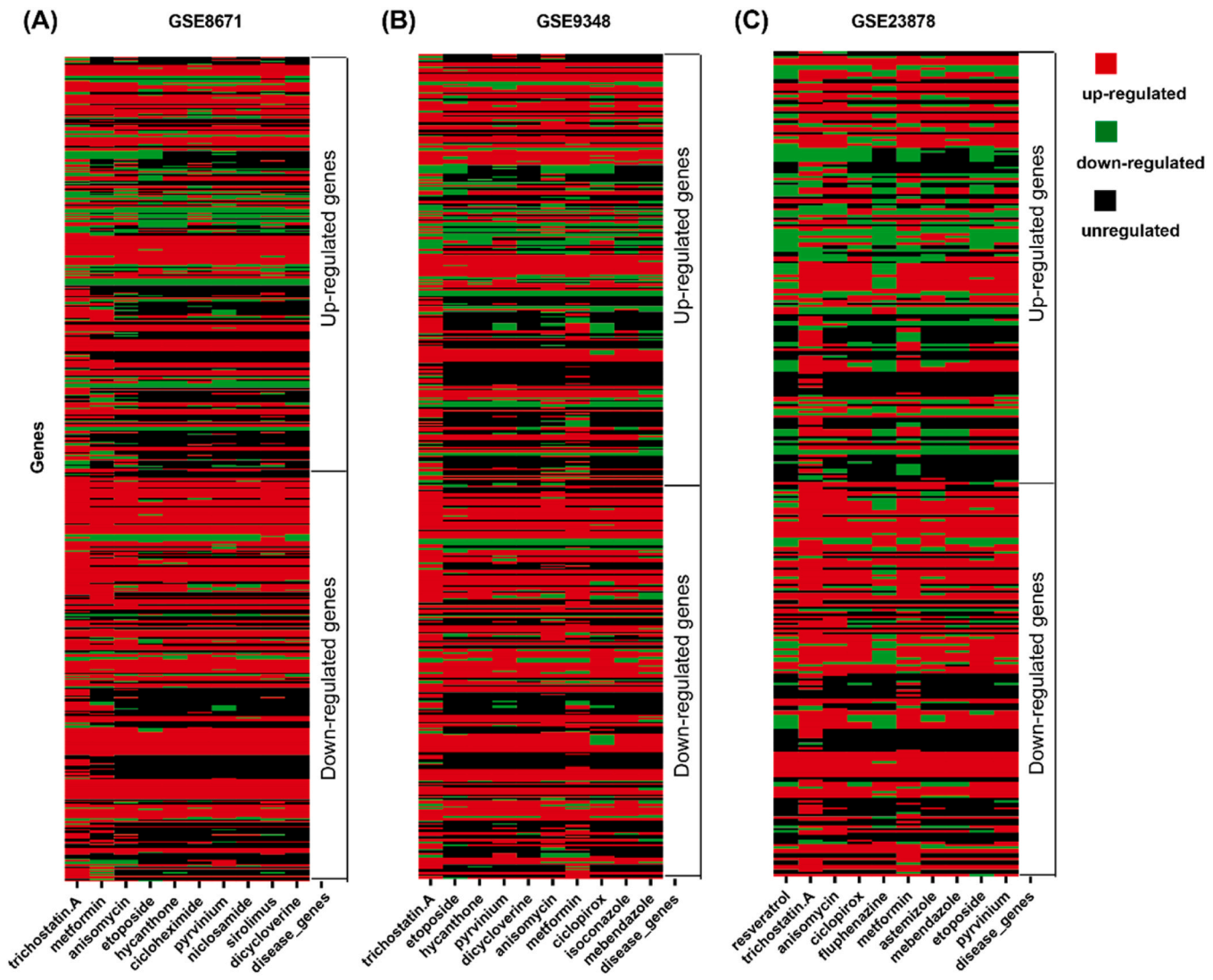

Fig. 5. The heatmap of the top 10 drugs for CRC in MCF7 cell line on three datasets, (A) GSE8671, (B) GSE9348, (C) GSE23878.

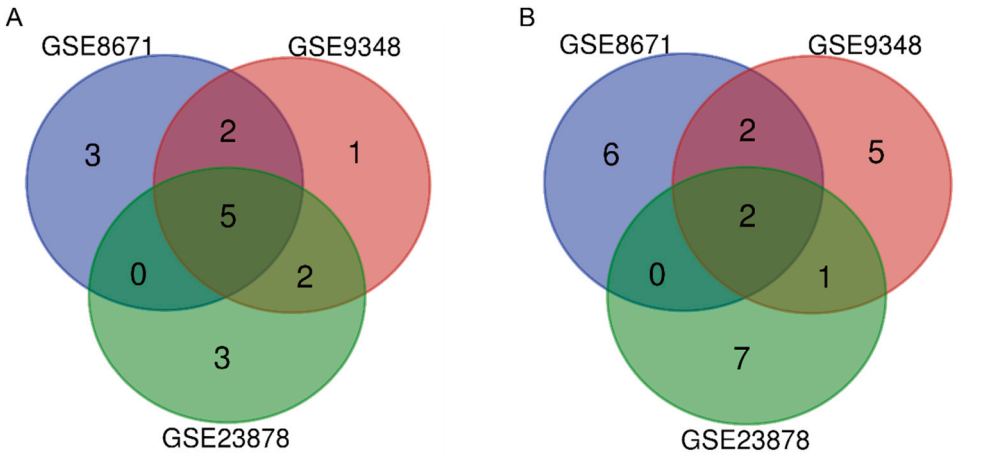

Fig. 6. Venn diagram of the top 10 drugs identified in the GSE8671, GSE9348, and GSE23878 datasets. (A) DRIE, (B) Hyper.

results showed that 90% of drugs had been validated by the CTD in colorectal, breast, and lung cancer, respectively. The associations between these top 10 potential drugs and three cancers were shown in Fig. 7. It showed that trichostatin.A, anisomycin, metformin, and pyrvinium are associated with three cancers by the CTD database. Furthermore, we identified several potential drugs that have new indications in Table 3. For example, Trichostatin.A (TSA) was a histone deacetylase inhibitor that could treat three cancers. Dai et al. [55] found

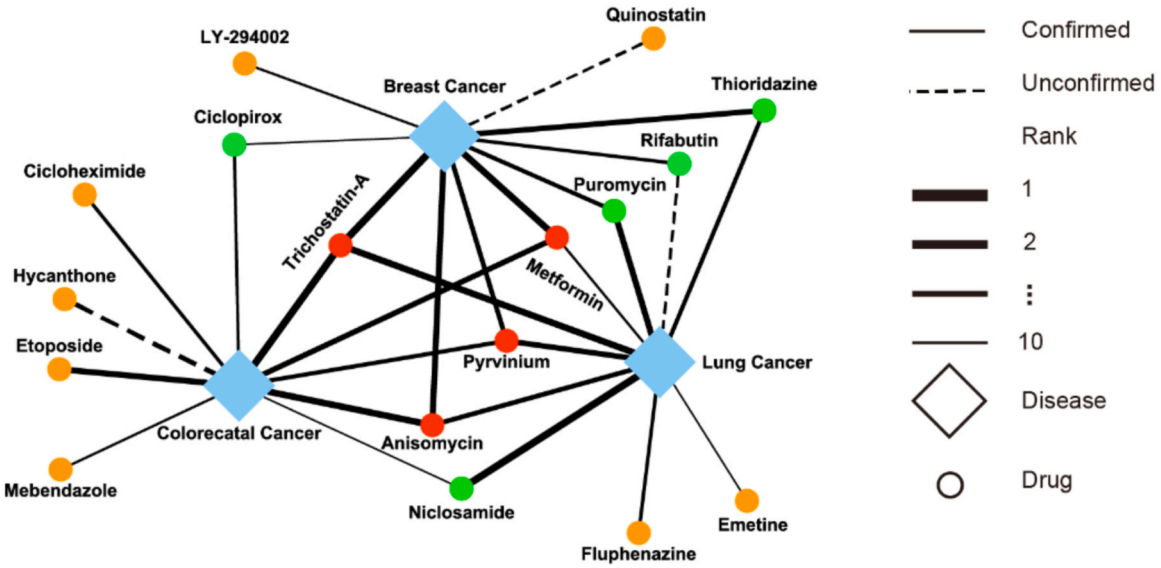

Fig. 7. The association network of the top 10 potential drugs for colorectal, breast, and lung cancer.

Table 3
Prioritization of drug repurposing candidates for cancer treatment based on DRIE.

Drug	Original Use	MOA	Targets	NI	Ref
Trichostati		HDAC inhibitor	HDAC1	CRC, BC, LC	[55,57,78]
Anisomycin	bacterial infections	DNA synthesis inhibitor	NHP2L1, RPL10L	CRC, BC, LC	[59]
Metformin	diabetes mellitus	insulin sensitizer	ACACB, PRKAB1,INS	CRC, BC, LC	[62,63]
Pyrvinium	pinworm (infectious disease)	androgen receptor antagonist	AR	CRC, BC, LC	[66]
Hycanthone	schistosomiasis (infectious disease)	RNA synthesis inhibitor (intercalate DNA)		Cancers	[71,72]
Quinostatin	bacterial infections	cellular S6 phosphorylation inhibitor		LC	[75]
Rifabutin	human immunodeficiency virus (HIV-1) (infectious disease)	protein synthesis inhibitor	CYP3A4	BC, LC	[77]

CRC: colorectal cancer, BC: breast cancer, LC: lung cancer, NI: new indication, MOA: Mode of Action

that TSA may induce endoplasmic reticulum stress via a p53-dependent mechanism in colon cancer cells. These findings offered valuable insights that can facilitate the development of therapeutic approaches aiming to harness the anticancer properties of TSA. Liu et al. [56] revealed that TSA exhibited effective inhibition of Grg1-induced lung tumorigenesis by down-regulating the expression of ErbB1 and ErbB2. Song et al. [57] presented that TSA inhibited the proliferation of triple-negative breast cancer cells through the induction of cell cycle arrest and apoptosis.

Anisomycin was a potent protein synthesis inhibitor, which prevented protein and DNA synthesis by inhibiting the peptidyl transferase 80 ribosome system. Ushijima et al. [58] found that the induction of GATA-6 dysfunction by anisomycin may hold promise as a potential chemotherapy strategy for colorectal cancer. Yang et al. [59] reported that anisomycin inhibited the cell growth of breast cancer via AMPK activation and subsequent downstream inhibition of the mTOR. Tan et al. [60] demonstrated that anisomycin had been shown to enhance the sensitivity of non-small-cell lung cancer cells to both chemotherapeutic agents and epidermal growth factor receptor (EGFR) inhibitors by suppressing the PI3K/Akt/mTOR signaling pathway.

Metformin, an oral biguanide, and hypoglycemic drug was the initial treatment for T2DM [61]. Several studies presented that metformin might be a candidate agent for the chemoprevention of colorectal cancer [62,63]. Kasznicki et al. [64] reported that metformin significantly reduced the morbidity of breast cancer in diabetic patients. The Warburg effect was well known and cancer cells exhibit very strong glucose uptake and metabolism and thereby prefer glycolysis. The primary feature of metformin was to reduce the level of glucose in the blood, thus reducing the source of energy for cancer cells. Moreover, metformin also affects the survival of cancer cells by downregulating the expression of the FAS gene, which was an essential gene for the fatty acid synthesis pathway.

Pyrvinium pamoate was a quinoline-derived anthranilic dye and an FDA-approved oral anthelmintic agent for the treatment of pinworm [65]. Furthermore, some studies had revealed that pyrvinium could inhibit tumor growth in some human cancers, including colon cancer [66], breast cancer [67], lung cancer [68], prostate cancer [69], and some hematological malignancies [70].

Furthermore, our method predicted that hycanthone, quinostatin, and rifabutin were associated with colorectal, breast, and lung cancer, respectively (Fig. 7). Although these drugs were not confirmed by CTD, some studies reported that these drugs were closely associated with other cancers [71–73].

Hycanthone was a medication that was used to treat parasitic worms, such as roundworms, hookworms, and tapeworms. Schutt et al. [71] found that a phase II study of hycanthone helps advanced colorectal carcinoma patients. Naidu et al. [72] reported that lucanthone and its derivative hycanthone inhibit APE1 to treat cancer.

Quinostatin was an antibiotic drug used to treat bacterial infections. Quinostatin was also regarded as an inhibitor of cellular S6 phosphorylation [73]. Kong et al. found that quinostatin had been predicted as a potential supplementary agent for the treatment of pediatric acute lymphoblastic leukemia [74]. Yang et al. [75] presented that quinostatin potently inhibited the mTOR signaling pathway by directly targeting the lipid-kinase activity of the catalytic subunits of class Ia PI3K. Dysregulation of the PI3K signaling pathway was associated with human cancer [76]. Thus, Quinostatin had been regarded as a potential cancer therapy by directly or indirectly modulating the PI3K signaling pathway.

Rifabutin was an antibiotic that inhibits DNA-dependent RNA polymerase activity in susceptible cells. It was confirmed to treat breast

Table 4
Performance comparison of all methods in the 11 datasets.

Dataset	Metrics	DRIE	CMAP	NP	Hyper
GSE8671	AUPR	**0.752**	0.508	0.6931	0.438
	AUROC	**0.669**	0.456	0.510	0.515
	Recall	**0.638**	0.225	0.266	0.634
	ACC	**0.650**	0.526	0.400	0.426
	F1	**0.695**	0.336	0.383	0.482
GSE9348	AUPR	**0.748**	0.498	0.695	0.412
	AUROC	**0.665**	0.392	0.486	0.476
	Recall	**0.659**	0.380	0.295	0.442
	ACC	**0.660**	0.580	0.413	0.580
	F1	**0.715**	0.503	0.413	0.461
GSE23878	AUPR	**0.807**	0.526	0.686	0.394
	AUROC	**0.702**	0.447	0.507	0.485
	Recall	**0.729**	0.192	0.295	0.483
	ACC	**0.690**	0.513	0.420	0.546
	F1	**0.750**	0.304	0.416	0.468
GSE29044	AUPR	**0.724**	0.479	0.614	0.362
	AUROC	**0.622**	0.410	0.500	0.422
	Recall	**0.804**	0.253	0.156	0.758
	ACC	**0.660**	0.573	0.380	0.500
	F1	**0.755**	0.384	0.243	0.555
GSE31448	AUPR	**0.680**	0.486	0.649	0.504
	AUROC	**0.642**	0.473	0.489	0.445
	Recall	0.585	0.093	0.195	**0.662**
	ACC	**0.630**	0.526	0.386	0.553
	F1	**0.635**	0.164	0.292	0.610
GSE42568	AUPR	**0.673**	0.475	0.648	0.404
	AUROC	**0.649**	0.452	0.487	0.454
	Recall	**0.607**	0.133	0.163	0.463
	ACC	**0.640**	0.546	0.380	0.533
	F1	**0.655**	0.227	0.256	0.469
GSE18842	AUPR	**0.823**	0.485	0.640	0.361
	AUROC	**0.714**	0.464	0.489	0.527
	Recall	**0.642**	0.223	0.148	0.600
	ACC	**0.660**	0.540	0.373	0.460
	F1	**0.710**	0.330	0.229	0.448
GSE19188	AUPR	**0.850**	0.471	0.640	0.499
	AUROC	**0.727**	0.424	0.490	0.585
	Recall	**0.676**	0.259	0.148	0.507
	ACC	**0.680**	0.573	0.373	0.426
	F1	**0.735**	0.384	0.229	0.448
GSE19804	AUPR	**0.849**	0.493	0.603	0.426
	AUROC	**0.732**	0.437	0.504	0.561
	Recall	**0.670**	0.202	0.139	0.550
	ACC	**0.660**	0.546	0.373	0.413
	F1	**0.720**	0.320	0.216	0.428
GSE55235	AUPR	0.534	0.409	**0.569**	0.460
	AUROC	0.565	**0.641**	0.495	0.596
	Recall	**0.780**	0.530	0.603	0.750
	ACC	**0.580**	0.580	0.550	0.570
	F1	**0.650**	0.597	0.608	0.644
GSE5281	AUPR	**0.611**	0.507	0.591	0.450
	AUROC	**0.573**	0.567	0.560	0.533
	Recall	**0.920**	0.771	0.603	0.883
	ACC	0.560	**0.630**	0.410	0.430
	F1	0.676	**0.704**	0.573	0.571

NP: Network proximity

cancer patients by CTD. Li et al. [77] showed that rifabutin inhibited the eIF4E-β-catenin axis in human lung cancer cells. This agent against lung cancer cells was effective in vitro cultured cells and in vivo xenograft mouse models by inhibiting proliferation and inducing apoptosis.

5.5. Comparison of DRIE with other drug repurposing methods

We compared the predictability of our DRIE method with other methods for drug repurposing: CMAP [15], Hyper [54], and Network proximity [28] on 11 datasets.

To evaluate the performance of DRIE, we conducted an extensive set of experiments on 11 datasets and compared DRIE with three existing approaches by AUPR, AUROC, Recall, ACC, and F1. According to Table 4, the results showed that DRIE was the best score based on AUPR, AUROC, Recall, ACC, and F1 in 10/11 datasets. Hence, our approach was more effective than other approaches. The results indicated that DRIE improved the prediction performance due to integrating gene expression profile and gene regulatory network.

6. Conclusion

Computational drug repurposing can identify new uses for approved drugs. It has a series of advantages such as cost-effectiveness and shortened timeline. In this paper, we proposed a method based on the inhibition score on the gene regulatory network. The key innovation of DRIE was applying gene regulatory network in the disease-related pathways to address the low overlap between drug-induced genes and disease-related genes.

First, to solve the issue of low overlap between drug-perturbed genes and disease-related genes, we constructed a disease-specific network based on disease-related pathways, which is an excellent complement to complete drug repurposing. Through integrating the pathway information, drug and disease's gene expression into a unified framework to improve the performance of DRIE. Then, we examined the performance of our model in different cell lines on eleven datasets. DRIE has the best capacity for prediction on the MCF7 cell line. To demonstrate the stability of our method, we predicted the top 10 drugs whose overlap percentage is greater than 50% in different datasets based on the CRC dataset. There were 9 of the top-10 DRIE-predicted agents were validated by the CTD in colorectal, breast, and lung cancer, respectively. It demonstrated that DRIE had a high practical predicting ability. Finally, to assess the performance of DRIE, we conducted an extensive set of experiments on 11 datasets, comparing with state-of-the-art approaches. DRIE achieved the best values by five evaluation metrics over 10/11 datasets. The results confirmed the efficacy of our approach.

Our study had several limitations. First, gene expression data MFC7 cell line may not fully represent the complexity of the tumor microenvironment or patient heterogeneity. Second, Computational drug repurposing method lack experimental validation of the identified drug candidates. Experimental studies, such as in vitro or in *vivo* assays, are necessary to validate efficacy of the predicted drug.

Funding

National Natural Science Foundation of China [62202115, 62072128, 62002079, 62102104], Open Project of Guangdong Provincial Key Laboratory of Artificial Intelligence in Medical Image Analysis and Application [2022B1212010011].

CRediT authorship contribution statement

Xianbin Li: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Writing – original draft, Writing –review & editing. **Minzhen Liao**: Conceptualization, Methodology, Writing – review & editing, Supervision. **Bing Wang**: Conceptualization, Writing – review & editing, Supervision. **Xiangzhen Zan**: Conceptualization, Writing – review & editing. **Yanhao Huo**: Conceptualization, Writing – review & editing. **Yue Liu**: Conceptualization, Writing – review & editing. **Zhenshen Bao**: Conceptualization, Writing – review & editing, Supervision, Project administration. **Peng Xu**: Conceptualization, Writing – review & editing, Supervision, Project administration. **Wenbin Liu**: Conceptualization, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as

influencing the position presented in, or the review of, the manuscript entitled, “A drug repurposing method based on inhibition effect on gene regulatory network”.

Data Availability

The implementation of DRIE is available at: https://github.com/eshinesimida/DRIE.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.09.007.

References

[1] McGuire AL, et al. The road ahead in genetics and genomics. *Nat Rev Genet* 2020; vol. 21(10):581–96. https://doi.org/10.1038/s41576-020-0272-6.

[2] Subbiah V. The next generation of evidence-based medicine. 2023/01/01 Nat Med 2023;vol. 29(1):49–58. https://doi.org/10.1038/s41591-022-02160-z.

[3] Sun D, Gao W, Hu H, Zhou S. Why 90% of clinical drug development fails and how to improve it? (Jul) *Acta Pharm Sin B* 2022;vol. 12(7):3049–62. https://doi.org/10.1016/j.apsb.2022.02.002.

[4] Hingorani AD, et al. Improving the odds of drug development success through human genomics: modelling study. 2019/12/11 Sci Rep 2019;vol. 9(1):18911. https://doi.org/10.1038/s41598-019-54849-w.

[5] Dimasi JA. New drug development in the United States from 1963 to 1999 (May) (Eng), *Clin Pharm Ther* 2001;vol. 69(5):286–96. https://doi.org/10.1067/mcp.2001.115132.

[6] Wouters OJ, McKee M, Luyten J. Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018. Mar 3 *Jama* 2020;vol. 323 (9):844–53. https://doi.org/10.1001/jama.2020.1166.

[7] Pushpakom S, et al. Drug repurposing: progress, challenges and recommendations (Jan) *Nat Rev Drug Discov* 2019;vol. 18(1):41–58. https://doi.org/10.1038/nrd.2018.168.

[8] Bagherian M, Sabeti E, Wang K, Sartor MA, Nikolovska-Coleska Z, Najarian K. Machine learning approaches and databases for prediction of drug-target interaction: a survey paper. Jan 18 *Brief Bioinforma* 2021;vol. 22(1):247–69. https://doi.org/10.1093/bib/bbz157.

[9] Su X, Hu L, You Z, Hu P, Wang L, Zhao B. A deep learning method for repurposing antiviral drugs against new viruses via multi-view nonnegative matrix factorization and its application to SARS-CoV-2. Jan 17 *Brief Bioinform* 2022;vol. 23(1). https://doi.org/10.1093/bib/bbab526.

[10] Hu P, et al. Learning from low-rank multimodal representations for predicting disease-drug associations. Nov 4 *BMC Med Inf Decis Mak* 2021;vol. 21(Suppl 1): 308. https://doi.org/10.1186/s12911-021-01648-x.

[11] Hu L, Zhang J, Pan X, Yan H, You ZH. HiSCF: leveraging higher-order structures for clustering analysis in biological networks. May 1 *Bioinformatics* 2021;vol. 37(4): 542–50. https://doi.org/10.1093/bioinformatics/btaa775.

[12] Shukla R, et al. Signature-based approaches for informed drug repurposing: targeting CNS disorders (Jan) *Neuropsychopharmacol* 2021;vol. 46(1):116–30. https://doi.org/10.1038/s41386-020-0752-6.

[13] Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. 2020/03/16 Cell Discov 2020;vol. 6(1):14. https://doi.org/10.1038/s41421-020-0153-3.

[14] Hernández-Lemus E, Martínez-García M. Pathway-based drug-repurposing schemes in cancer: the role of translational bioinformatics. *Front Oncol* 2020;vol. 10:605680. https://doi.org/10.3389/fonc.2020.605680.

[15] Lamb J, et al. The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease. Sep 29 *Science* 2006;vol. 313(5795):1929–35. https://doi.org/10.1126/science.1132939.

[16] Lamb J. The connectivity map: a new tool for biomedical research (Jan) *Nat Rev Cancer* 2007;vol. 7(1):54–60. https://doi.org/10.1038/nrc2044.

[17] Napolitano F, et al. Drug repositioning: a machine-learning approach through data integration. Jun 22 *J Chemin-* 2013;vol. 5(1):30. https://doi.org/10.1186/1758-2946-5-30.

[18] Chen YW, et al. PharmOmics: a species- and tissue-specific drug signature database and gene-network-based drug repositioning tool. Apr 15 *Isc* 2022;vol. 25(4): 104052. https://doi.org/10.1016/j.isci.2022.104052.

[19] Dudley JT, et al. Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Aug 17 *Sci Transl Med* 2011;vol. 3(96):96ra76. https://doi.org/10.1126/scitranslmed.3002648.

[20] Cui C, et al. Drug repurposing against breast cancer by integrating drug-exposure expression profiles and drug–drug links based on graph neural network. Bioinformatics 2021;vol. 37(18):2930–7. https://doi.org/10.1093/bioinformatics/btab191.

[21] Saberian N, Peyvandipour A, Donato M, Ansari S, Draghici S. "A new computational drug repurposing method using established disease-drug pair knowledge," (in eng). Mar 6 Bioinformatics 2019. https://doi.org/10.1093/bioinformatics/btz156.

[22] Sirota M, et al. Discovery and preclinical validation of drug indications using compendia of public gene expression data. Aug 17 *Sci Transl Med* 2011;vol. 3(96): 96ra77. https://doi.org/10.1126/scitranslmed.3001318.

[23] Yu L, Huang J, Ma Z, Zhang J, Zou Y, Gao L. "Inferring drug-disease associations based on known protein complexes. *BMC Med Genom* 2015;vol. 8 Suppl 2(Suppl 2): S2. https://doi.org/10.1186/1755-8794-8-s2-s2.

[24] Peyvandipour A, Saberian N, Shafi A, Donato M, Draghici S. A novel computational approach for drug repurposing using systems biology. Aug 15 *Bioinformatics* 2018; vol. 34(16):2817–25. https://doi.org/10.1093/bioinformatics/bty133.

[25] Zhang W, Yue X, Huang F, Liu R, Chen Y, Ruan C. Predicting drug-disease associations and their therapeutic function based on the drug-disease association bipartite network. Aug 1 *Methods* 2018;vol. 145:51–9. https://doi.org/10.1016/j.ymeth.2018.06.001.

[26] Liu H, Song Y, Guan J, Luo L, Zhuang Z. Inferring new indications for approved drugs via random walk on drug-disease heterogenous networks. Dec 23 *BMC Bioinform* 2016;vol. 17(Suppl 17):539. https://doi.org/10.1186/s12859-016-1336-7.

[27] Zhao BW, Hu L, You ZH, Wang L, Su XR. HINGRL: predicting drug-disease associations with graph representation learning on heterogeneous information networks. Dec 9 *Brief Bioinform* 2021. https://doi.org/10.1093/bib/bbab515.

[28] Cheng F, et al. Network-based approach to prediction and population-based validation of in silico drug repurposing. Jul 12 *Nat Commun* 2018;vol. 9(1):2691. https://doi.org/10.1038/s41467-018-05116-5.

[29] Guney E, Menche J, Vidal M, Barabasi AL. Network-based in silico drug efficacy screening. Feb 1 *Nat Commun* 2016;vol. 7:10331. https://doi.org/10.1038/ncomms10331.

[30] Hansen J, et al. Systems pharmacology-based integration of human and mouse data for drug repurposing to treat thoracic aneurysms. Jun 6 *JCI Insight* 2019;vol. 4(11). https://doi.org/10.1172/jci.insight.127652.

[31] Li J, Lu Z. "Pathway-based drug repositioning using causal inference. *BMC Bioinform* 2013;vol. 14 Suppl 16(Suppl 16):S3. https://doi.org/10.1186/1471-2105-14-s16-s3.

[32] Napolitano F, et al. gene2drug: a computational tool for pathway-based rational drug repositioning. May *Bioinformatics* 2018;vol. 34(9):1498–505. https://doi.org/10.1093/bioinformatics/btx800.

[33] Yu L, Wang B, Ma X, Gao L. The extraction of drug-disease correlations based on module distance in incomplete human interactome. Dec 23 *BMC Syst Biol* 2016;vol. 10(Suppl 4):111. https://doi.org/10.1186/s12918-016-0364-2.

[34] Yu L, et al. Prediction of novel drugs for hepatocellular carcinoma based on multi-source random walk (Jul-Aug) *IEEE/ACM Trans Comput Biol Bioinform* 2017;vol. 14 (4):966–77. https://doi.org/10.1109/tcbb.2016.2550453.

[35] Yu L, Zhao J, Gao L. Predicting potential drugs for breast cancer based on miRNA and tissue specificity. *Int J Biol Sci* 2018;vol. 14(8):971–82. https://doi.org/10.7150/ijbs.23350.

[36] Yu L, et al. Predicting therapeutic drugs for hepatocellular carcinoma based on tissue-specific pathways. *PLoS Comput Biol* 2021;vol. 17(2):e1008696. https://doi.org/10.1371/journal.pcbi.1008696.

[37] Li X, Shen L, Shang X, Liu W. Subpathway analysis based on signaling-pathway impact analysis of signaling pathway. 07/24, 11/24/received, 06/18/accepted PLoS One 2015;vol. 10(7):e0132813. https://doi.org/10.1371/journal.pone.0132813.

[38] Han X, Kong Q, Liu C, Cheng L, Han J. SubtypeDrug: a software package for prioritization of candidate cancer subtype-specific drugs. Jan 18 *Bioinformatics* 2021. https://doi.org/10.1093/bioinformatics/btab011.

[39] Nam S, et al. "PATHOME-Drug: a subpathway-based poly-pharmacology drug-repositioning method," (in eng). Sep 13 Bioinformatics 2021. https://doi.org/10.1093/bioinformatics/btab566.

[40] Davis AP, et al. The Comparative Toxicogenomics Database's 10th Year Anniversary: update 2015. no. Database issue, pp. D914-20, Jan *Nucleic Acids Res* 2015;vol. 43. https://doi.org/10.1093/nar/gku935.

[41] Sabates-Bellver J, et al. Transcriptome profile of human colorectal adenomas. *Mol Cancer Res* 2007;vol. 5(12):1263–75. https://doi.org/10.1158/1541-7786.mcr-07-0267.

[42] Hong Y, Downey T, Eu KW, Koh PK, Cheah PY. "A 'metastasis-prone' signature for early-stage mismatch-repair proficient sporadic colorectal cancer patients and its implications for possible therapeutics," (in eng). Clin Exp Metastas 2010;vol. 27(2): 83–90. https://doi.org/10.1007/s10585-010-9305-4.

[43] Uddin S, et al. Genome-wide expression analysis of Middle Eastern colorectal cancer reveals FOXM1 as a novel target for cancer therapy. *Am J Pathol* 2011;vol. 178(2):537–47. https://doi.org/10.1016/j.ajpath.2010.10.020.

[44] Sabatier R, et al. Down-regulation of ECRG4, a candidate tumor suppressor gene, in human breast cancer. *PloS One* 2011;vol. 6(11):e27656. https://doi.org/10.1371/journal.pone.0027656.

[45] Clarke C, et al. Correlating transcriptional networks to breast cancer survival: a large-scale coexpression analysis. *Carcinogenesis* 2013;vol. 34(10):2300–8. https://doi.org/10.1093/carcin/bgt208.

[46] Colak D, et al. Age-specific gene expression signatures for breast tumors and cross-species conserved potential cancer progression markers in young women. *PloS One* 2013;vol. 8(5):e63204. https://doi.org/10.1371/journal.pone.0063204.

[47] Sanchez-Palencia A, et al. Gene expression profiling reveals novel biomarkers in nonsmall cell lung cancer. Jul 15 *J Cancer J Int du Cancer* 2011;vol. 129(2):355–64. https://doi.org/10.1002/ijc.25704.

[48] Hou J, et al. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. Apr 22 *PloS One* 2010;vol. 5(4):e10312. https://doi.org/10.1371/journal.pone.0010312.

[49] Lu TP, et al. Identification of regulatory SNPs associated with genetic modifications in lung adenocarcinoma. Mar 24 *BMC Res Notes* 2015;vol. 8:92. https://doi.org/10.1186/s13104-015-1053-8.
[50] Woetzel D, et al. Identification of rheumatoid arthritis and osteoarthritis patients by transcriptome-based rule set generation. Apr 1 *Arthritis Res Ther* 2014;vol. 16 (2):R84. https://doi.org/10.1186/ar4526.
[51] Liang WS, et al. Gene expression profiles in anatomically and functionally distinct regions of the normal aged human brain. Feb 12 *Physiol Genom* 2007;vol. 28(3): 311–22. https://doi.org/10.1152/physiolgenomics.00208.2006.
[52] Ritchie ME, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;vol. 43(7):e47. https://doi.org/10.1093/nar/gkv007.
[53] Debrabant B, Soerensen M. Gene set analysis for GWAS: assessing the use of modified Kolmogorov-Smirnov statistics. *Stat Appl Genet Mol Biol* 2014;vol. 13(5): 553–66. https://doi.org/10.1515/sagmb-2013-0015.
[54] Jia Z, Liu Y, Guan N, Bo X, Luo Z, Barnes MR. Cogena, a novel tool for co-expressed gene-set enrichment analysis, applied to drug repositioning and drug mode of action discovery. *BMC Genom* 27 2016;vol. 17:414. https://doi.org/10.1186/s12864-016-2737-8.
[55] Dai L, He G, Zhang K, Guan X, Wang Y, Zhang B. Trichostatin A induces p53-dependent endoplasmic reticulum stress in human colon cancer cells. *Oncol Lett* 2019;vol. 17(1):660–7. https://doi.org/10.3892/ol.2018.9641.
[56] Liu J, et al. Trichostatin A suppresses lung adenocarcinoma development in Grg1 overexpressing transgenic mice. Aug 7 *Biochem Biophys Res Commun* 2015;vol. 463 (4):1230–6. https://doi.org/10.1016/j.bbrc.2015.06.090.
[57] Song X, Wu JQ, Yu XF, Yang XS, Yang Y. Trichostatin A inhibits proliferation of triple negative breast cancer cells by inducing cell cycle arrest and apoptosis. Nov 15 *Neoplasm* 2018;vol. 65(6):898–906. https://doi.org/10.4149/neo_2018_181212N476.
[58] Ushijima H, Horyozaki A, Maeda M. Anisomycin-induced GATA-6 degradation accompanying a decrease of proliferation of colorectal cancer cell. Sep 9 *Biochem Biophys Res Commun* 2016;vol. 478(1):481–5. https://doi.org/10.1016/j.bbrc.2016.05.139.
[59] Yang W, Zhou C, Sun Q, Guan G. Anisomycin inhibits angiogenesis, growth, and survival of triple-negative breast cancer through mitochondrial dysfunction, AMPK activation, and mTOR inhibition. Jul 1 *Can J Physiol Pharm* 2022;vol. 100(7): 612–20. https://doi.org/10.1139/cjpp-2021-0577.
[60] Tan H, Hu B, Xie F, Zhu C, Cheng Z. Anisomycin sensitizes non-small-cell lung cancer cells to chemotherapeutic agents and epidermal growth factor receptor inhibitor via suppressing PI3K/Akt/mTOR. *Fundam Clin Pharm* 2021;vol. 35(5): 822–31. https://doi.org/10.1111/fcp.12641.
[61] Madsen KS, Chi Y, Metzendorf MI, Richter B, Hemmingsen B. Metformin for prevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus. (Eng), *Cochrane Database Syst Rev* 2019;vol. 12(12):Cd008558. https://doi.org/10.1002/14651858.CD008558.pub2.
[62] Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. *Gastroenterol* 2004;vol. 127(4):1044–50. https://doi.org/10.1053/j.gastro.2004.07.011.
[63] Zhang ZJ, et al. Reduced risk of colorectal cancer with metformin therapy in patients with type 2 diabetes: a meta-analysis. *Diabetes Care* 2011;vol. 34(10): 2323–8. https://doi.org/10.2337/dc11-0512.
[64] Kasznicki J, Sliwinska A, Drzewoski J. Metformin in cancer prevention and therapy. *Ann Transl Med* 2014;vol. 2(6):57. https://doi.org/10.3978/j.issn.2305-5839.2014.06.01.
[65] Turner JA, Johnson Jr PE. Pyrvinium pamoate in the treatment of pinworm infection (enterobiasis) in the home. *Pediatr* 1962;vol. 60:243–51. https://doi.org/10.1016/s0022-3476(62)80042-0.
[66] Li B, et al. Repurposing the FDA-approved pinworm drug pyrvinium as a novel chemotherapeutic agent for intestinal polyposis. *PloS One* 2014;vol. 9(7):e101969. https://doi.org/10.1371/journal.pone.0101969.
[67] Serra V, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Oct 1 *Cancer Res* 2008;vol. 68(19):8022–30. https://doi.org/10.1158/0008-5472.Can-08-1385.
[68] Feng J, et al. Blocking STAT3 by pyrvinium pamoate causes metabolic lethality in KRAS-mutant lung cancer. " (Eng), *Biochem Pharmacol* 2020;vol. 177:113960. https://doi.org/10.1016/j.bcp.2020.113960.
[69] Lim M, et al. Ligand-independent and tissue-selective androgen receptor inhibition by pyrvinium. Mar 21 (Eng), *ACS Chem Biol* 2014;vol. 9(3):692–702. https://doi.org/10.1021/cb400759d.
[70] Xiang W, et al. Pyrvinium selectively targets blast phase-chronic myeloid leukemia through inhibition of mitochondrial respiration. Oct 20 *Oncotarget* 2015;vol. 6(32): 33769–80. https://doi.org/10.18632/oncotarget.5615.
[71] Schutt AJ, Dalton RJ, Kovach JS, Moertel CG, O'Connell MJ. "Phase II study of hycanthone in patients with advanced colorectal carcinoma," (in eng) (Jun) Cancer Treat Rep 1983;vol. 67(6):593–4 (Jun).
[72] Naidu MD, et al. Lucanthone and its derivative hycanthone inhibit apurinic endonuclease-1 (APE1) by direct protein binding. (Eng), *PloS One* 2011;vol. 6(9): e23679. https://doi.org/10.1371/journal.pone.0023679.
[73] Sato S, Murata A, Shirakawa T, Uesugi M. Biochemical target isolation for novices: affinity-based strategies. Jun 25 (Eng), *Chem Biol* 2010;vol. 17(6):616–23. https://doi.org/10.1016/j.chembiol.2010.05.015.
[74] Kong L, Zhang X, Li C, Zhou L. Potential therapeutic targets and small molecular drugs for pediatric B-precursor acute lymphoblastic leukemia treatment based on microarray data. *Oncol Lett* 2017;vol. 14(2):1543–9. https://doi.org/10.3892/ol.2017.6343.
[75] Yang J, Shamji A, Matchacheep S, Schreiber SL. Identification of a small-molecule inhibitor of class Ia PI3Ks with cell-based screening. *Chem Biol* 2007;vol. 14(4): 371–7. https://doi.org/10.1016/j.chembiol.2007.02.004.
[76] Bader AG, Kang S, Zhao L, Vogt PK. Oncogenic PI3K deregulates transcription and translation. *Nat Rev Cancer* 2005;vol. 5(12):921–9. https://doi.org/10.1038/nrc1753.
[77] Li J, Huang Y, Gao Y, Wu H, Dong W, Liu L. Antibiotic drug rifabutin is effective against lung cancer cells by targeting the eIF4E-β-catenin axis. Apr 1 *Biochem Biophys Res Commun* 2016;vol. 472(2):299–305. https://doi.org/10.1016/j.bbrc.2016.02.120.
[78] Tang D, et al. Trichostatin A reverses the chemoresistance of lung cancer with high IGFBP2 expression through enhancing autophagy. Mar 2 *Sci Rep* 2018;vol. 8(1): 3917. https://doi.org/10.1038/s41598-018-22257-1.