Identification of putative drugs for gastric adenocarcinoma utilizing differentially expressed genes and connectivity map

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Abstract. Gastric adenocarcinoma (GAC) is a challenging disease with dim prognosis even after surgery; hence, novel treatments for GAC are in urgent need. The aim of the present study was to explore new potential compounds interfering with the key pathways related to GAC progression. The differentially expressed genes (DEGs) between GAC and adjacent tissues were identified from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) database. Connectivity Map (CMap) was performed to screen candidate compounds for treating GAC. Subsequently, pathways affected by compounds were overlapped with those enriched by the DEGs to further identify compounds which had anti-GAC potential. A total of 843 DEGs of GAC were identified. Via Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, 13 pathways were significantly enriched. Moreover, 78 compounds with markedly negative correlations with DEGs were revealed in CMap database ($P<0.05$ and Enrichment <0). Subpathways of cell cycle and p53 signaling pathways, and core genes of these compounds, cyclin B1 (CCNB1) and CDC6, were identified. This study further revealed seven compounds that may be effective against GAC; in particular methylbenzethonium chloride and alexidine have never yet been reported for GAC treatment. In brief, the candidate drugs identified in this study may provide new options to improve the treatment of patients with GAC. However, the biological effects of these drugs need further investigation.

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Abbreviations: GAC, gastric adenocarcinoma; CMap, connectivity map; TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; FC, fold change; GO, Gene Ontology; PPI, protein-protein interaction; HD, Hirschsprung disease; NAG, non-steroidal anti-inflammatory drug-activated gene; FOXM1, forkhead box transcription factor 1

Key words: differentially expressed genes, pathways, connectivity map, gastric adenocarcinoma

Introduction

Globally, gastric cancer is the fifth leading cause of cancer and the third leading cause of death from cancer (1,2). In 2015, 679,100 new cases of gastric cancer were diagnosed in China, accounting for 15.8% of the total number of newly occurred cancer cases. In addition, gastric cancer resulted in 498,000 deaths, 17.7% of all cancer-related deaths, and the incidence of gastric cancer has been steadily increasing (3). Among these cases, gastric adenocarcinoma (GAC) accounts for 95% of all gastric cancer cases. Research indicates that, even after surgery, the outcome of GAC patients remains dim (4-6). Therefore, other novel treatments for GAC should be developed. The study of small-molecule drugs aiming at multiple protein pathways modulating tumor progression, invasion, and metastasis formation, has received much interest in recent years (7-9). The purpose of this study was to discover new, potential small-molecule drugs by using multiple online databases.

Connectivity Map (CMap) is one of the gene expression profile databases used to process the genetic data. CMap was developed by Lamb and his colleagues from Broad Institute of MIT, Whitehead Institute and Harvard Medical School, (Boston, MA, USA) (10). CMap utilizes the differential gene expression of human cells which are treated with small-molecule drugs, to construct a biological application database based on connection of small-molecule drugs, gene expression and different diseases. CMap allows scholars of drug development to take advantage of gene expression profiling data and, therefore, identify the drugs highly correlated with disease, infer the main chemical structure of most drug molecules, and summarize the mechanism of possible action of drug molecules.

To explore new drugs for GAC, based on the integrated subpathway analysis, we implemented an *in silico* method

for the reuse of GAC drugs. First, we identified the differentially expressed genes (DEGs) between GAC and non-tumor tissues identified in The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases, and then determined the potential pathways affecting the progression of GAC. Next, CMap was used to verify the pathways of GAC affected by small-molecule treatment. Finally, small-molecule drugs that can target subpathways related to GAC were considered as potential new agents in the treatment of GAC (Fig. 1). The candidate drugs identified in our approach may provide a new direction for improving the treatment of patients with GAC.

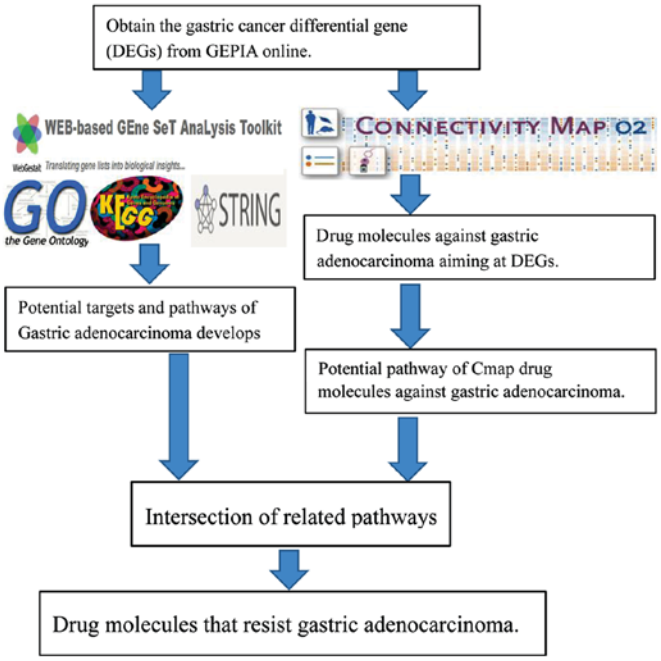

Figure 1. The flow chart of the study process.

Materials and methods

DEG analysis of GAC. Using the GEPIA online analysis website (http://gepia.cancer-pku.cn/), the expression data of mRNA of GAC in TCGA and GTEx databases were performed with the value of fold change (FC). Among these data, only the genes with logFC >2 and logFC <-2 were defined as DEGs, including upregulated and downregulated ones.

Enrichment analysis of DEGs. DEGs were performed with Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis with the WebGestalt database (http://www.webgestalt.org/). Also, pathway analysis was conducted by Gene List Analysis (http://www.pantherdb.org/) to obtain possible pathways during the development of GAC. Finally, we used the STRING database (https://string-db.org/) to analyze the protein-protein interaction (PPI) of the ultimate DEGs as previously reported (11-16). In this study, GO outcomes were analyzed visibly with Cytoscape software (version 3.7.0, U.S. National Institute of General Medical Sciences (NIGMS), https://cytoscape.org/).

CMap for DEG analysis of drug molecule cures for GAC. The CMap database (https://portals.broadinstitute.org/CMap/) (build 02) contains over 7,000 gene expression profiles and 1,309 chemicals. To analyze this potential mechanism for the development of GAC, we first set up the files in query signature format for DEGs obtained from the TCGA (https://cancergenome.nih.gov/) and GTEx databases (https://gtexportal.org/home/). We then entered the CMap quick query interface to import the files of upregulated and downregulated genes and ran them with CMap analysis. In this way, we analyzed the drug molecules for the DEGs of GAC (17). The negatively related drugs (P<0.05 and Enrichment <0) for anti-GAC were then screened.

Correlation data between drug molecules and subpathways. The chip expression profiles of 1,309 drugs and the genes affected by the drugs using the CMap database were downloaded. Furthermore, we identified the subpathways that obtain significant enrichment for each small-molecule drug with the affected genes according to the method reported by a previous publication (18). Consistent with the reference, 196 small-molecular drugs and 104 subpathways were also achieved. The overlapped pathways between those from CMap and those enriched by DEGs were determined, which were identified as potential pathways related to both the treatment and pathogenesis of GAC. Finally, the drug-pathway network was constructed for GAC.

Results

Screening results of DEGs. Altogether, 843 DEGs in mRNA expression of GAC were obtained, which included 638 upregulated genes and 205 downregulated ones. The next analysis was based on this screening result.

Functional annotation, pathway enrichment and PPI network analysis. Through GO analysis, in the annotations of biological progress, the top three most significant processes were mitotic sister chromatid segregation, mitotic cell cycle and nuclear division. In the terms of cellular component, the top most significant annotations were extracellular space, chromosome, centromeric region and spindle. As for analysis of molecular function, the top three most significant functions were serine hydrolase activity, chemokine activity and serine-type peptidase activity (Table I and Fig. 2). KEGG pathway analysis indicated that DEGs were obviously centralized in 13 pathways, including cell cycle, protein digestion and absorption, *Staphylococcus aureus* infection, and the p53 signaling pathway (Table II and Fig. 3). From the PPI network analysis, we acquired the following hub genes: *CCNB1, AURKA, CDC6, KIF11, OIP5, NCAPG, KIF23, DLGAP5 and NDC80* (nodes ≥100) (Fig. 4).

CMap analysis to achieve potential compounds for GAC. The 843 DEGs of GAC mentioned above led to 78 compounds by CMap (Table III) when P<0.05 and Enrichment <0.

Intersection of small-molecule drug correlative pathways and KEGG pathways. According to a previous method (18), we performed subpathway analysis and obtained

Table I. Top 10 of the most significantly enriched GO terms.

Pathway ID	Terms	Gene count	FDR	P-value
BP				
GO:0000070	Mitotic sister chromatid segregation	36	0	0
GO:0000278	Mitotic cell cycle	112	0	0
GO:0000280	Nuclear division	76	0	0
GO:0000819	Sister chromatid segregation	47	0	0
GO:0007049	Cell cycle	142	0	0
GO:0007059	Chromosome segregation	58	0	0
GO:0007067	Mitotic nuclear division	69	0	0
GO:0008283	Cell proliferation	165	0	0
GO:0022402	Cell cycle process	125	0	0
GO:0042127	Regulation of cell proliferation	127	0	0
CC				
GO:0005615	Extracellular space	129	0	0
GO:0000775	Chromosome, centromeric region	34	1.14E-13	2.22E-16
GO:0005819	Spindle	42	1.52E-13	4.44E-16
GO:0000793	Condensed chromosome	35	2.86E-13	1.11E-15
GO:0098687	Chromosomal region	42	2.54E-12	1.23E-14
GO:0000779	Condensed chromosome, centromeric region	25	2.88E-12	1.68E-14
GO:0000776	Kinetochore	26	1.18E-11	8.04E-14
GO:0000777	Condensed chromosome kinetochore	23	1.39E-11	1.20E-13
GO:0009986	Cell surface	64	1.39E-11	1.21E-13
GO:0005694	Chromosome	71	1.40E-10	1.36E-12
MF				
GO:0017171	Serine hydrolase activity	30	2.76E-07	1.77E-10
GO:0008009	Chemokine activity	14	2.76E-07	3.88E-10
GO:0008236	Serine-type peptidase activity	29	2.76E-07	5.77E-10
GO:0004252	Serine-type endopeptidase activity	27	2.76E-07	6.04E-10
GO:0042379	Chemokine receptor binding	15	3.08E-07	8.91E-10
GO:0004175	Endopeptidase activity	42	3.08E-07	1.01E-09
GO:0045236	CXCR chemokine receptor binding	9	4.33E-07	1.66E-09
GO:0001664	G-protein coupled receptor binding	27	6.08E-05	2.66E-07
GO:0032395	MHC class II receptor activity	6	7.59E-05	3.74E-07
GO:0042802	Identical protein binding	82	1.18E-04	6.44E-07

GO, Gene Ontology; BP, biological progress; CC, cellular component; MF, molecular function; FDR, false discovery rate.

104 subpathways. After integrating these 104 subpathways with 13 KEGG pathways generated by the DEGs, two pathways related to anti-GAC drug molecules were finally achieved (Table IV and Fig. 5), including cell cycle and p53 signaling pathways. These two pathways were related to 32 genes and seven CMap small-molecule drugs. The genes involved in these two KEGG pathway were *CDKN2A, DBF4, CHEK1, ORC6, SFN, MAD2L1, MCM2, MCM4, MCM5, PCNA, PLK1, CCND1, BUB1, BUB1B, TTK, CDC45, CCNA2, CCNB1, PKMYT1, CCNB2, PTTG1, ESPL1, CDK1, CDC6, CDC20, CDC25C, IGFBP3, GTSE1, SERPINB5, RPRM, RRM2 and BID*. The PPI analysis with the above 32 genes demonstrated two hub genes (*CCNB1 and CDC6*). The seven CMap small-molecule drugs were troglitazone, methylbenzethonium chloride, thiostrepton, alexidine, vorinostat, methotrexate and etoposide (Fig. 6).

Expression levels of CCNB1 and CDC6 mRNA in GAC tissues. The expression levels of *CCNB1* and *CDC6* mRNA in GACs were queried from GEPIA database (http://gepia.cancer-pku.cn/). The results showed that the two genes were both highly expressed in GAC tissues compared to non-cancerous gastric tissues (Fig. 7).

Verification of predicting small-molecule drugs of GAC with online literature retrieval. Using PubMed, we identified studies that investigated the effect of relevant drugs on GAC. We found 268 articles related to the effect of methotrexate on

Table II. Significantly enriched KEGG pathway.

Pathway ID	Terms	Gene count	FDR	P-value
hsa04110	Cell cycle	26	2.83E-08	9.34E-11
hsa04974	Protein digestion and absorption	17	1.33E-04	8.80E-07
hsa05150	*Staphylococcus aureus* infection	12	9.58E-04	9.49E-06
hsa04115	p53 signaling pathway	13	1.35E-03	1.79E-05
hsa05140	Leishmaniasis	12	9.11E-03	1.50E-04
hsa05323	Rheumatoid arthritis	13	1.40E-02	3.07E-04
hsa04610	Complement and coagulation cascades	12	1.40E-02	3.24E-04
hsa05416	Viral myocarditis	10	1.56E-02	4.13E-04
hsa05310	Asthma	7	1.73E-02	5.12E-04
hsa05164	Influenza A	18	4.47E-02	1.63E-03
hsa04512	ECM-receptor interaction	11	4.47E-02	1.64E-03
hsa04060	Cytokine-cytokine receptor interaction	24	4.47E-02	1.77E-03
hsa04640	Hematopoietic cell lineage	12	4.86E-02	2.09E-03

KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

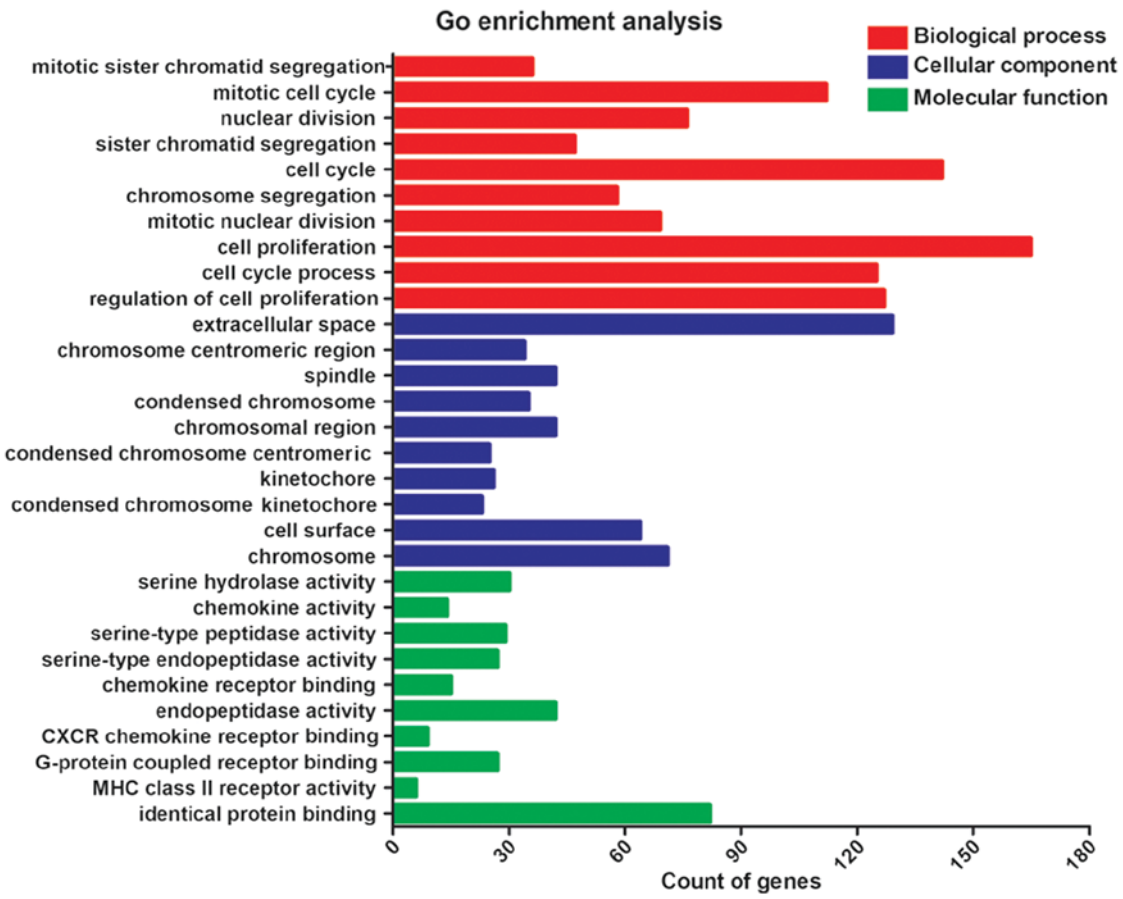

Figure 2. Gene Ontology (GO) enrichment analysis of the differentially expressed genes in gastric adenocarcinoma.

GAC, 403 articles related to etoposide, and 17 articles related to troglitazone, which is a diabetes drug that may inhibit GAC. Nine studies concerned vorinostat and three studies were related to thiostrepton. Most importantly, methylbenzethonium chloride and alexidine have never been addressed in the literature of GAC.

Discussion

In the present study, we identified DEGs of GAC and found several pathways and hub genes that may play a critical role in the pathogenesis and development of GAC. Also, through

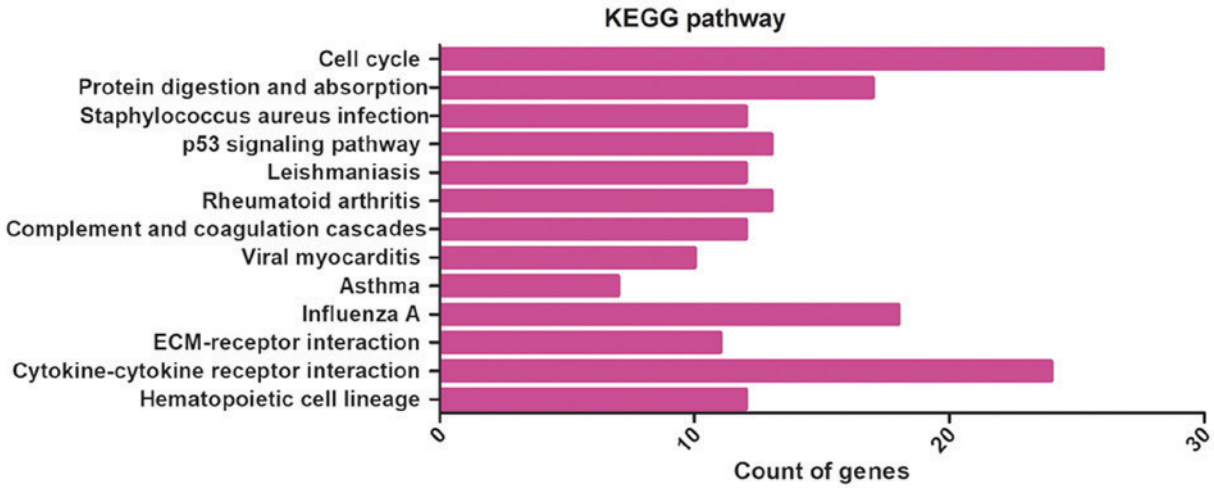

Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the differentially expressed genes in gastric adenocarcinoma.

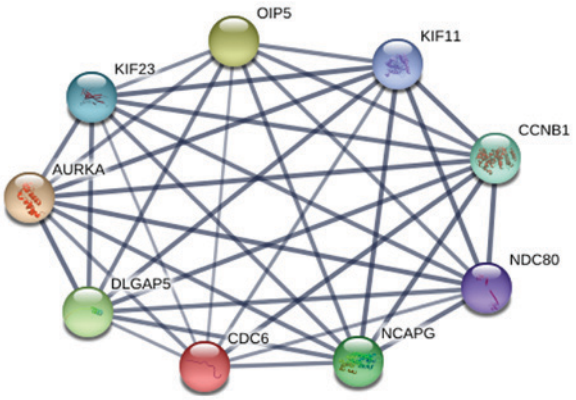

Figure 4. The top 9 hub genes with most interaction lines in protein-protein interaction (PPI) analysis.

the connectivity mapping approach, some known compounds were found to share similar pathways of those generated from the DEGs of GAC, including methotrexate, etoposide, troglitazone, thiostrepton, vorinostat, methylbenzethonium chloride and alexidine. The findings from the present study suggest that methylbenzethonium chloride and alexidine could act as novel potential drugs for the treatment of GAC and warrant further investigation, as they have never been tested previously.

The CMap database reveals the connection between disease, genes and drugs, using gene expression data and the 'similarity' concept with a small-molecular compound or the gene expression spectrum of the drug as the core (19). CMap database provides a unique method for drug development through comparison to filter candidate compounds curing diseases, and it has been adopted by several scholars (20,21). For instance, Xiao *et al* used gene expression profile chip technology and the CMap database to study molecular mechanisms of Hirschsprung disease (HD) and potential drugs. They found differences in the neuronal developmental disorders of HD genes and signaling pathways, and discovered that some compounds may offset the damage of HD development (22).

In this study, the DEGs between GAC and adjacent tissues were compared with the expression profiles in CMap to identify negatively correlative compounds that are potential compounds for GAC. Among the candidate compounds determined in the present study, two compounds (alexidine and methylbenzethonium) are particularly important. Alexidine is an antimicrobial agent with high affinity for bacteria, which can be used in the root canal irrigation solution of oral treatment (23). Feng *et al*, using high-throughput drug screening tests, identified that alexidine is an antitumor drug that can inhibit cytokines and growth factors necessary for multiple myeloma (24). Meanwhile, methylbenzethonium chloride, a broad spectrum antibiotic, was found to be able to specifically induce apoptosis in undifferentiated embryonic stem cells of mice (25). The effect could be applied to prevent reoccurrence of the tumor after stem cell transplantation therapy. Methylbenzethonium chloride may become another novel anticancer agent (25).

The present study showed that alexidine had the lowest connectivity score (-0.996), indicating a highly negative correlation with the DEGs of GAC. The connectivity score of methylbenzethonium chloride also suggests that it has the capacity to inhibit the growth of GAC. In addition, this study predicted that both alexidine and methylbenzethonium chloride can play a vital role in inhibiting GAC by regulating the p53 signaling pathway. Previous studies have shown that the p53 signaling pathway regulates various cellular functions, including apoptosis, induction of aging, and inhibition of cell growth, migration and invasion (26-28). However, the specific molecular mechanisms of alexidine and methylbenzethonium chloride for antitumor activity need to be further explored.

Five other compounds achieved in the present study have been mentioned in other studies. Troglitazone hinders BGC-823 GAC cell proliferation and promotes its apoptosis by inducing expression of the non-steroidal anti-inflammatory drug-activated gene (NAG) (29). In addition, thiostrepton was found to reverse drug resistance in GAC by inhibiting the forkhead box transcription factor 1 (FOXM1) (30). Vorinostat (31), methotrexate (32) and etoposide (33) are proven to inhibit the proliferation of GAC cells. This evidence indicates that the predictive method in this study is convincing and worth being used for drug exploration.

Table III. CMap compounds matched by the DEGs of gastric adenocarcinoma.

Rank	CMap name	Cell line	N	Enrichment	P-value	Specificity	Percent non-null
1	Phenoxybenzamine	MCF7	3	-0.984	0	0	100
2	Vorinostat	MCF7	7	-0.844	0	0.1262	100
3	Trichostatin A	PC3	55	-0.705	0	0.1149	96
4	Trichostatin A	MCF7	92	-0.59	0	0.1881	88
5	Trichostatin A	HL60	34	-0.465	0	0.1946	52
6	LY-294002	MCF7	34	-0.454	0	0.1625	70
7	Resveratrol	MCF7	6	-0.865	0.00002	0.0082	100
8	Alexidine	PC3	2	-0.996	0.00004	0	100
9	15-Delta prostaglandin J2	MCF7	8	-0.695	0.00018	0.0414	87
10	Meticrane	PC3	2	-0.991	0.00026	0	100
11	Astemizole	PC3	2	-0.99	0.00026	0.0192	100
12	Thiostrepton	MCF7	2	-0.973	0.00141	0.0283	100
13	Clemizole	PC3	2	-0.973	0.00141	0	100
14	Sulconazole	MCF7	2	-0.973	0.00157	0	100
15	Mefloquine	PC3	2	-0.971	0.00167	0.0431	100
16	MG-262	PC3	2	-0.968	0.00223	0.0738	100
17	Cloperastine	PC3	2	-0.968	0.00223	0.0149	100
18	Thioridazine	PC3	5	-0.736	0.0027	0.102	100
19	Methotrexate	MCF7	3	-0.877	0.00379	0.0853	100
20	Valproic acid	HL60	14	-0.448	0.00403	0.2883	64
21	Cloperastine	MCF7	3	-0.873	0.00415	0.0196	100
22	Fludroxycortide	PC3	2	-0.954	0.00453	0.0171	100
23	Pyrantel	PC3	2	-0.946	0.00644	0.0144	100
24	Thioguanosine	MCF7	2	-0.945	0.00658	0.0455	100
25	6-Bromoindirubin-3'-oxime methylbenzethonium	PC3	4	-0.755	0.00732	0.0498	100
26	Chloride	PC3	2	-0.939	0.00767	0.0598	100
27	Chlorpromazine	PC3	4	-0.749	0.0079	0.0168	100
28	Vorinostat	HL60	3	-0.839	0.00837	0.1705	100
29	Vitexin	MCF7	2	-0.936	0.00861	0.0051	100
30	Acetazolamide	MCF7	2	-0.931	0.00984	0	100
31	Pyrvinium	MCF7	4	-0.731	0.0105	0.1304	100
32	5224221	MCF7	2	-0.927	0.01097	0.1429	100
33	Methacholine chloride	MCF7	2	-0.924	0.01181	0.0278	100
34	Cortisone	MCF7	2	-0.921	0.01262	0.0117	100
35	Carbachol	MCF7	2	-0.919	0.01318	0.0058	100
36	Clotrimazole	MCF7	3	-0.807	0.01444	0.0556	100
37	Dipyridamole	MCF7	3	-0.799	0.01671	0.04	100
38	Abamectin	MCF7	2	-0.907	0.01746	0.05	100
39	LY-294002	PC3	12	-0.423	0.01802	0.3669	66
40	Troglitazone	PC3	4	-0.696	0.01804	0.1159	100
41	Luteolin	MCF7	2	-0.904	0.01839	0.0476	100
42	Hydroflumethiazide	MCF7	2	-0.902	0.01913	0.0601	100
43	Homochlorcyclizine	MCF7	2	-0.898	0.02066	0.0968	100
44	Gemfibrozil	PC3	2	-0.896	0.02167	0.0208	100
45	Withaferin A	PC3	2	-0.894	0.02223	0.0917	100
46	Tanespimycin	PC3	12	-0.414	0.02239	0.3382	58
47	Prochlorperazine	MCF7	9	-0.472	0.0231	0.1892	66
48	Ciclosporin	MCF7	4	-0.679	0.02349	0.0576	75
49	Disulfiram	PC3	2	-0.891	0.02382	0.0667	100
50	Procaine	PC3	2	-0.89	0.024	0.0294	100
51	0173570-0000	PC3	4	-0.677	0.02407	0.1349	75

Table III. Continued.

Rank	CMap name	Cell line	N	Enrichment	P-value	Specificity	Percent non-null
52	Tretinoin	MCF7	13	-0.395	0.02531	0.3655	61
53	Fluphenazine	PC3	3	-0.769	0.02534	0.1026	100
54	Loperamide	MCF7	3	-0.767	0.026	0.087	100
55	Dilazep	PC3	2	-0.886	0.02612	0.0784	100
56	Trifluoperazine	PC3	3	-0.765	0.02656	0.1379	100
57	3-Acetylcoumarin	MCF7	3	-0.764	0.02692	0.022	100
58	Flunarizine	MCF7	2	-0.884	0.02712	0.068	100
59	Sulfaguanidine	PC3	2	-0.878	0.02972	0.0202	100
60	Ethaverine	MCF7	2	-0.878	0.03004	0.0133	100
61	Amiodarone	MCF7	3	-0.754	0.03043	0.1039	100
62	Picotamide	PC3	2	-0.875	0.03127	0.0162	100
63	Felodipine	MCF7	5	-0.594	0.0318	0.1376	80
64	Prestwick-1084	MCF7	2	-0.873	0.03201	0.0545	100
65	Monobenzone	MCF7	2	-0.871	0.03306	0.0548	100
66	Pioglitazone	PC3	5	-0.586	0.03585	0.3436	60
67	Levocabastine	MCF7	2	-0.866	0.03626	0.0615	100
68	Noretynodrel	MCF7	2	-0.865	0.03628	0.0822	100
69	Trifluoperazine	MCF7	9	-0.448	0.03655	0.2308	55
70	15-Delta prostaglandin J2	HL60	3	-0.738	0.03684	0.1429	100
71	Etoposide	MCF7	2	-0.864	0.03712	0.1	100
72	Bufexamac	MCF7	2	-0.863	0.0376	0.0556	100
73	0179445-0000	PC3	4	-0.644	0.03853	0.0685	75
74	15-Delta prostaglandin J2	PC3	3	-0.734	0.03856	0.1507	100
75	Minaprine	PC3	2	-0.858	0.04008	0.031	100
76	Oxymetazoline	PC3	2	-0.855	0.04181	0.0345	100
77	Nortriptyline	MCF7	2	-0.852	0.04338	0.0901	100
78	CP-690334-01	MCF7	4	-0.633	0.04418	0.1027	50
79	SB-203580	PC3	2	-0.85	0.04515	0.0464	100
80	Scriptaid	PC3	2	-0.849	0.04537	0.1596	100
81	Esculetin	MCF7	2	-0.848	0.04609	0.0671	100
82	Fluspirilene	MCF7	2	-0.848	0.0464	0.1748	100
83	Sulfadoxine	MCF7	2	-0.845	0.04829	0.0481	100
84	Monorden	PC3	5	-0.562	0.04932	0.106	60
85	Ivermectin	MCF7	2	-0.843	0.04937	0.1404	100
86	Norethisterone	MCF7	2	-0.842	0.04994	0.0263	100

CMap, Connectivity Map; DEGs, differentially expressed genes. N, number of all instances of the same perturbagen made in the same cell line. A total of 78 compounds were included, among which, four compounds were administered to two different cell lines and two compounds were administered to three different cell lines. Thus, there are 86 rows in the table.

In this study, we used bioinformatic methods to screen differentially expressing potential genetic biomarkers based on RNA-seq data. The results of pathway enrichment analysis indicated 13 pathways which were evidently enriched with DEGs, including the cell cycle, protein digestion and absorption, *Staphylococcus aureus* infection and the p53 signaling pathway. In addition, these DEGs were analyzed with CMap and subpathways, and two (cell cycle and p53 signaling pathway) were found to be closely related to the treatment potential and occurrence of GAC. *CCNB1* and *CDC6* in these pathways were also hub genes in the PPI network.

The clinical role of these hub genes was analyzed also based on publicly available RNA-seq data, and it was found that CCNB1 was upregulated in patients with GAC. CCNB1 is a member of the cell cycle protein B family; it is a regulatory protein involved in mitosis, mostly expressed in the G2/M period, and plays a significant role in the S-to-G2/M phases (34). Therefore, overexpression of CCNB1 in GAC leads to chaos in the cell cycle, mitosis promotion and cell proliferation. Previous research has shown that silencing of CDKN3 stimulates cell cycle arrest by reducing the expression of CDK2, CDC25, CCNB1 and CCNB2 in human GAC

Table IV. CMap negatively correlated compounds matched by pathway.

Drug name	Pathway name	Subpathway ID
Alexidine	p53 signaling pathway	path:04115_2; path:04115_1; path:04115_7
Mefloquine	Toll-like receptor signaling pathway	path:04620_17; path:04620_18; path:04620_22; path:04620_9
Mefloquine	Steroid hormone biosynthesis	path:00140_3; path:00140_19; path:00140_16; path:00140_8
Astemizole	Toll-like receptor signaling pathway	path:04620_12; path:04620_9; path:04620_18; path:04620_17
Thiostrepton	p53 signaling pathway	path:04115_1
Methotrexate	p53 signaling pathway	path:04115_7; path:04115_1; path:04115_4; path:04115_3; path:04115_2
Sulconazole	Metabolism of xenobiotics by cytochrome P450	path:00980_3
Resveratrol	Tryptophan metabolism	path:00380_5
Resveratrol	Toxoplasmosis	path:05145_18
Thioguanosine	Steroid hormone biosynthesis	path:00140_7; path:00140_8
MG-262	Steroid hormone biosynthesis	path:00140_1; path:00140_9; path:00140_8; path:00140_6; path:00140_5
Methylbenzethonium chloride	p53 signaling pathway	path:04115_1
Monobenzone	MAPK signaling pathway	path:04010_30
Trifluoperazine	Protein processing in endoplasmic reticulum	path:04141_18: path:04141_1
5224221	Steroid hormone biosynthesis	path:00140_18; path:00140_27; path:00140_9; path:00140_8; path:00140_4
Vitexin	Steroid hormone biosynthesis	path:00140_19
Disulfiram	Protein processing in endoplasmic reticulum	path:04141_1
Thioridazine	Pathways in cancer	path:05200_29; path:05200_18; path:05200_11
Vorinostat	p53 signaling pathway	path:04115_1; path:04115_2; path:04115_4; path:04115_3
Etoposide	p53 signaling pathway	path:04115_7; path:04115_1; path:04115_3
Withaferin A	Steroid hormone biosynthesis	path:00140_25; path:00140_5; path:00140_10; path:00140_4
Pyrvinium	Steroid hormone biosynthesis	path:00140_6; path:00140_16; path:00140_19; path:00140_17; path:00140_18; path:00140_4
Scriptaid	Steroid hormone biosynthesis	path:00140_9; path:00140_6; path:00140_17; path:00140_16; path:00140_5; path:00140_1
Trichostatin A	Steroid hormone biosynthesis	path:00140_10; path:00140_19; path:00140_6; path:00140_8; path:00140_9
0173570-0000	Steroid hormone biosynthesis	path:00140_16; path:00140_4; path:00140_17; path:00140_3; path:00140_6; path:00140_10; path:00140_18; path:00140_13; path:00140_7; path:00140_8
Troglitazone	Cell cycle	path:04110_17
Prochlorperazine	Protein processing in endoplasmic reticulum	path:04141_1
LY-294002	Steroid hormone biosynthesis	path:00140_6; path:00140_27
Tanespimycin	MAPK signaling pathway	path:04010_15
Monorden	Steroid hormone biosynthesis	path:00140_3; path:00140_7; path:00140_18

CMap, connectivity map.

cells, thus, inhibits the proliferation of tumor cells (35). It was found *in vivo* that dipalmitoyl phosphatidic acid could dramatically inhibit the growth of tumors in a mouse subcutaneous tumor model, and suppress cell proliferation and

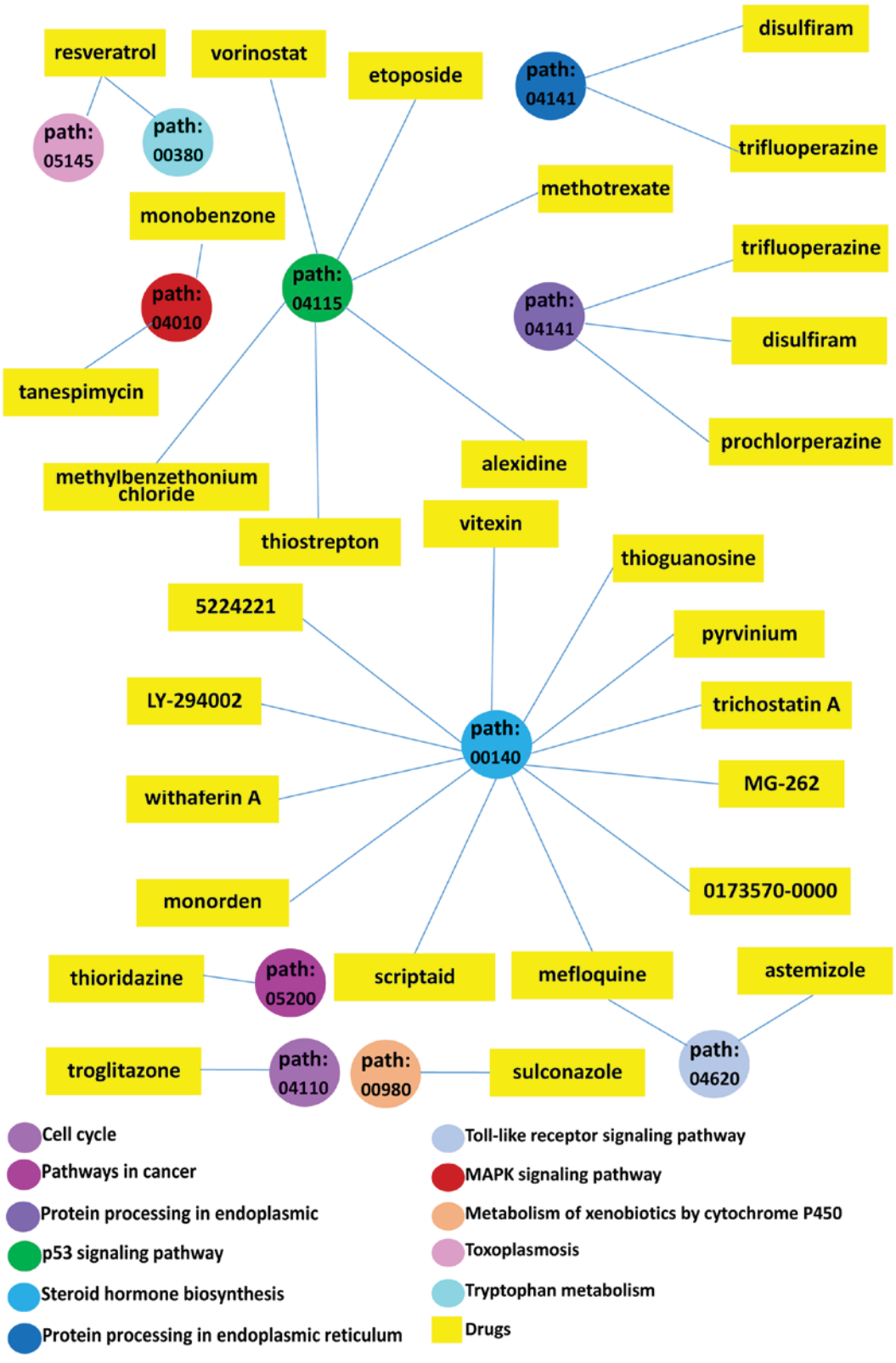

Figure 5. Small-molecular drugs and their perturbed pathways in gastric adenocarcinoma.

angiogenesis in triple-negative breast cancer. The suppressing effect was mediated partly due to reduction in the expression of CCNB1 (36). Therefore, CCNB1 may be an important target gene in the treatment of GAC, and the present study predicted that compounds aimed at this target gene may be reasonable and effective in treating GAC. Recent studies have shown that knockdown of CDC6 expression levels can interfere with the cell cycle and inhibit the proliferation of prostate and ovarian cancer cells (37,38). This evidence suggests that CDC6 may also be a potential biomarker for GAC therapy.

The present study comprehensively analyzed the possible mechanism of treating GAC by data mining in the public gene

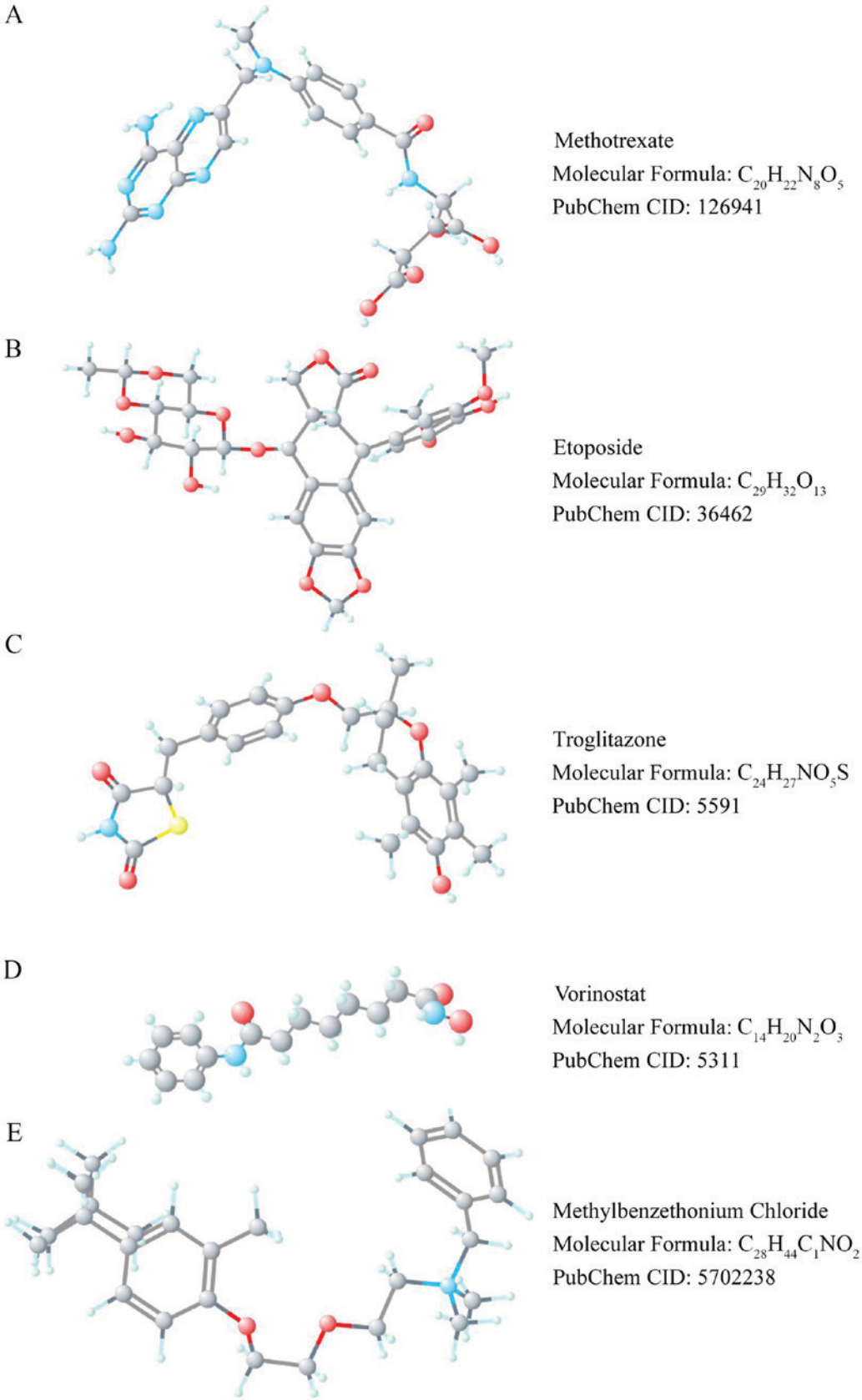

Figure 6. The 3D conformers of the five compounds that counteracted the molecular signature effect in gastric adenocarcinoma. The 3D structures of the five compounds were provided by PubChem (https://pubchem.ncbi.nlm.nih.gov/compound). (A) Methotrexate, (B) etoposide, (C) troglitazone, (D) vorinostat and (E) methylbenzethonium chloride.

chip databases and bioinformatic analyses. We discovered cell cycle and p53 signaling pathways and key gene targets CCNB1 and CDC6 as potential targets of GAC treatment. We further predicted that seven known compounds may be effective in

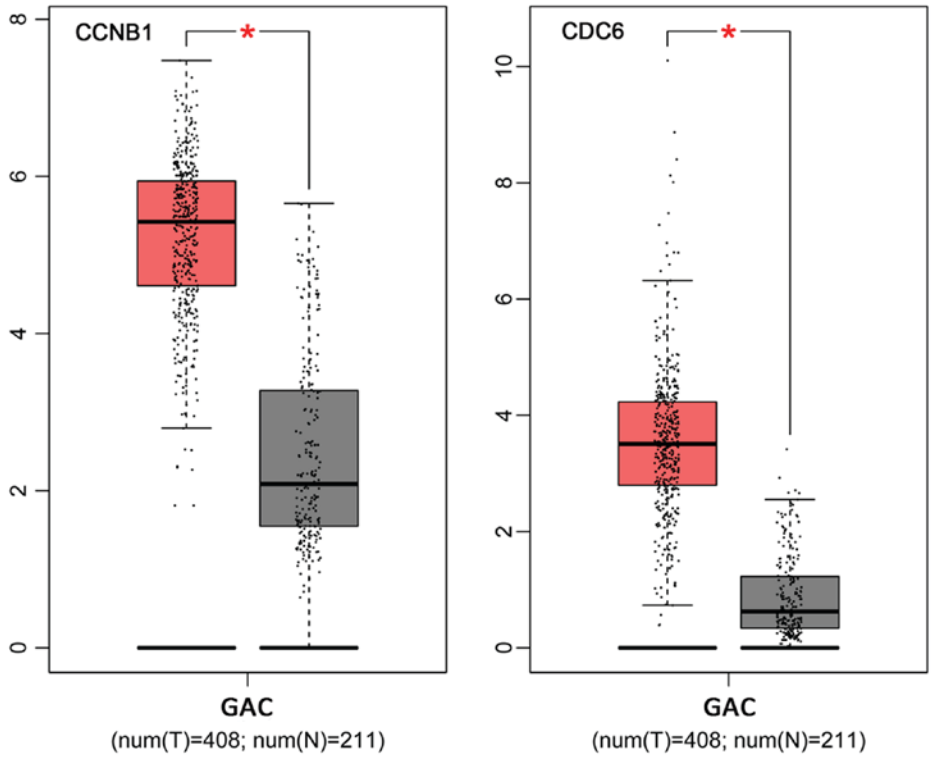

Figure 7. Verification of CCNB1 and CDC6 mRNA expression levels. The data were provided by GEPIA database based on 408 GAC (T; red) and 211 controls (N; grey). GAC, gastric adenocarcinoma, *P<0.05.

curing GAC, including methylbenzethonium chloride and alexidine, which have never been previously reported to treat GAC. However, several limitations should be admitted. Firstly, the current findings were based on *in silico* methods and validations are certainly needed. Secondly, CMap did not cover GAC cell lines and only provided general DEGs post treatment of existing drugs. The overlapping pathways of DEGs from TCGA and pathways from Cmap also need to be confirmed. Thirdly, the precise mechanism of the drugs we recommended remains to be investigated. Hence, further clinical, *in vitro* and *in vivo* experiments are needed to verify the definite effects and molecular mechanism of the potential drugs on GAC.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

ZXC, XPZ, HQY, RZ and JSP analyzed and interpreted the data and wrote the draft of the manuscript. XGQ, RQH, JM, ZBF, GC and TQG conceived and designed the study, supervised the data mining, corrected and revised the draft. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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