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

ZU-XUAN CHEN¹, XIAO-PING ZOU², HUANG-QUN YAN², RUI ZHANG², JIN-SHU PANG², XIN-GAN QIN³, RONG-QUAN HE⁴, JIE MA⁴, ZHEN-BO FENG², GANG CHEN² and TING-QING GAN¹

¹Department of Medical Oncology, The Second Affiliated Hospital of Guangxi Medical University; Departments of ²Pathology, ³Gastrointestinal Surgery and ⁴Medical Oncology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China

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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.

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.

Materials and methods

**DEG analysis of GAC.** Using the GEPIA online analysis website (http://geopia.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, NCPAG, 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...
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...
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

| 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.
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 recurrence 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   | Fludroxyctide              | 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 methylbenzethionium | 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   | Vite Xin                   | 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   | Prochloperazine            | 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               |
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.

| 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  | -0.866     | 0.02612 | 0.0784      | 100              |
| 56   | Triluoperazine| 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| PC3    | 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   | Levocarbastine| MCF7   | 2  | -0.866     | 0.03626 | 0.0615      | 100              |
| 68   | Noretyndrel | MCF7      | 2  | -0.865     | 0.03628 | 0.0822      | 100              |
| 69   | Triluoperazine| 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   | Esceulain   | 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.
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

cells, thus, inhibits the proliferation of tumor cells (35). It

| 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                                                                 |
| Disutiliram          | 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_5                      |
| 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                     |
| 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_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.
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
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
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.

References

1. Wippel HH, Santos MDM, Clasen MA, Kurt LU, Nogueira FCS, Carvalho CE, McCormick TM, Neto GPB, Alves LR, da Gloria da Costa Carvalho M, et al: Comparing intestinal versus diffuse gastric cancer using a PEFF-oriented proteomic pipeline. J Proteomics 171: 63-72, 2018.
2. Li W, Song D, Li H, Liang L, Zhao N and Liu T: Reduction in peripheral CD19+CD24hiCD27+ B cell frequency predicts favourable clinical course in XELOX-treated patients with advanced gastric cancer. Cell Physiol Biochem 41: 2045-2052, 2017.
3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
4. Costa NR, Gil da Costa RM and Medeiros R: A viral map of gastrointestinal cancers. Life Sci 199: 188-200, 2018.
profiling coupled with connectivity map database mining reveals relationships with breast cancer causing or preventing properties.

Busby J, Murray L, Mills K, Zhang SD, Liberante F and Gajadien T, van der Leije CS, van Kerkwijk A, Eijken M, Brum AM, van de Peppel J, Nguyen L, Pancorbo M, Sangiuliano BA, Betsch T, Dang YW, Lin P, Liu LM, He RQ, Li XJ, Liang L, Sun M, Si G, Sun HS and Si FC: Inhibition of CREPT restrains gastric cancer growth by regulation of cycle arrest, migration and apoptosis via ROS-regulated p53 pathway. Biochem Biophys Res Commun 496: 1183-1190, 2018.

Wang C, Wang J and Bai P: Troglitazone induces apoptosis in gastric cancer cells through the NAG-1 pathway. Mol Med 23: 4634-4642, 2018.

Sun M, Si G, Sun HS and Si FC: Inhibition of CREPT restrains gastric cancer growth by regulation of cycle arrest, migration and apoptosis via ROS-regulated p53 pathway. Biochem Biophys Res Commun 496: 1183-1190, 2018.

Wang C, Wang J and Bai P: Troglitazone induces apoptosis in gastric cancer cells through the NAG-1 pathway. Mol Med 23: 4634-4642, 2018.