Could microtubule inhibitors be the best choice of therapy in gastric cancer with high immune activity: mutant DYNC1H1 as a biomarker

Jin Bai¹²³, BoWen Yang¹²³, Ruichuan Shi¹²³, Xincheng Shao¹²³, Xinye Shao¹²³, Yujing Yang¹²³, Fang Wang¹²³, Jiawen Xiao⁵, Xiujuan Qu¹²³, Yunpeng Liu¹²³, Ye Zhang⁶, Zhi Li¹²³

¹Department of Medical Oncology, The First Hospital of China Medical University, Shenyang 110001, China  
²Key Laboratory of Anticancer Drugs and Biotherapy of Liaoning Province, The First Hospital of China Medical University, Shenyang 110001, China  
³Liaoning Province Clinical Research Center for Cancer, Shenyang 110001, China  
⁴Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, Ministry of Education, Shenyang 110001, China  
⁵Laboratory I of Cancer Institute, The First Hospital of China Medical University, Shenyang 110001, China  
⁶Laboratory I of Cancer Institute, The First Hospital of China Medical University, Shenyang 110001, China

Correspondence to: Zhi Li, Ye Zhang; email: zli@cmu.edu.cn, yzhang21@cmu.edu.cn  
Keywords: immune checkpoint blockade, microtubule inhibitors, mutation, DYNC1H1, gastric cancer  
Received: January 13, 2020  Accepted: August 27, 2020  Published: November 20, 2020

ABSTRACT

Immune checkpoint blockade (ICB) has achieved unprecedented breakthroughs in various cancers, including gastric cancer (GC) with high immune activity (MSI-H or TMB-H), yet clinical benefits from ICB were moderate. Here we aimed to identify the most appropriate drugs which can improve outcomes in GC. We firstly compared MSI-H and TMB-H GC samples with normal samples in TCGA-STAD cohort, respectively. After that, Connectivity Map database repurposed nine candidate drugs (CMap score < 90). Then, microtubule inhibitors (MTIs) were screened as the significant candidate drugs with their representative gene sets strongly enriched (p < 0.05) via GSEA. GDSC database validated higher activities of some MTIs in GC cells with MSI-H and TMB-H (p < 0.05). Furthermore, some MTIs activities were positively associated with mutant Dynene Cytoplasmic 1 Heavy Chain 1 (DYNC1H1) (p < 0.05) based on NCI-60 cancer cell line panel. DYN1H1 was high frequently alteration in GC and was positively associated with TMB-H and MSI-H. Mutant DYN1H1 may be accompanied with down-regulation of MTIs-related genes in GC or change the binding pocket to sensitize MTIs. Overall, this study suggested that some MTIs may be the best candidate drugs to treat GC with high immune activity, especially patients with DYN1H1 mutated.

INTRODUCTION

High immune activity is one of the essential characteristics of malignant tumors [1]. Currently, as the advent of immune checkpoint blockade (ICB), GC patients with microsatellite instability-high (MSI-H) exhibited sensitivity to ICB in multiple clinical trials [2–4]. Tumors with high tumor mutational burden (TMB-H) also showed better responses to ICB and more prolonged overall survival than those with low TMB in gastric cancer [5]. Therefore, MSI-H and TMB-H can be considered as the high immune activity biomarkers in GC to repurpose better drugs that can improve clinical outcomes.

ICB has achieved overwhelming breakthroughs in the treatment of different malignant tumors such as GC [6, 7]. ICB significantly prolonged the overall survival of GC patients [2, 4, 8, 9]. However, the therapeutic benefit was limited to specific subgroups such as MSI-
Supplementary Figure 1
ted that ERCC1 and DPD
hich target the high immune activity
ork
es from the Molecular Signatures
y subgroups for ICB treatment
ropolitan pathways (like
MICROTUBULE_BINDING”,

www.aging
were shown in Supplementary Table 1).
939 common down
a total of 539 up
patients were TMB
samples displayed MSI
To identify DEGs in GC with high immune activity, we
GC (N = 375) and adjacent normal tissues (N =
samples displayed MSI-H, and 15.7% (118/375) of GC
indeed, DEGs were screened
from each group compared with normal tissues. Overall,
at a total of 539 up-regulated and 1353 down-regulated
genes were found in the MSI-H group, 632 up-regulated
and 979 down-regulated genes were screened from
TMB-H group. Together, there were 1375 common
DEGs in the two groups: 436 common up-regulated and 939 common down-regulated genes (common DEGs
were shown in Supplementary Table 1).

RESULTS
Identification of differentially expressed genes
(DEGs) in gastric cancer with high immune activity
To identify DEGs in GC with high immune activity, we
used the GC (N = 375) and adjacent normal tissues (N =
of the TCGA-STAD cohort. 12.5% (47/375) GC
samples displayed MSI-H, and 15.7% (118/375) of GC
patients were TMB-H. Indeed, DEGs were screened
from each group compared with normal tissues. Overall,
at a total of 539 up-regulated and 1353 down-regulated
genes were found in the MSI-H group, 632 up-regulated
and 979 down-regulated genes were screened from
TMB-H group. Together, there were 1375 common
DEGs in the two groups: 436 common up-regulated and 939 common down-regulated genes (common DEGs
were shown in Supplementary Table 1).

Identification of high immune activity targeting
candidate drugs for gastric cancer
We firstly used DEGs of MSI-H and TMB-H groups to
query in the CMap database respectively to repurpose
candidate drugs. CMap results revealed nine drugs both
from the two groups were significantly negatively
correlated with the DEGs (connectivity score < -0.90).
These nine drugs can be classified into seven types
(Figure 1A) according to the annotation information
of the CMap database and previous studies [18–23].

We next sought to the significant candidate drugs. We
screened the representative functional gene sets of the
seven drug types from the Molecular Signatures
Database (MSigDB) (Supplementary Table 2). Based
on the common DEGs, GSEA was performed with
representative functional gene sets for each drug type.
GSEA results showed that some microtubule inhibitors-
related pathways: "GO_MICROTUBULE_BINDING", "GO_MICROTUBULE_DEPOLYMERIZATION” were
significantly enriched (p-value < 0.05) in DEGs of high
immune activity, while other types of drug showed no
statistical significance (p-value > 0.05) (Figure 1B).

Furthermore, we utilized the genomics of drug sensitivity
in cancer (GDSC) database to validate the anticancer
sensitivities of MTIs in GC with high immune activity. A
total of 13 tubulin related drugs (such as MTIs, AURK
inhibitors, and KIF inhibitors) and 26 gastric cancer cells
were available in the GDSC database. For the TMB levels
and MSI status in GC cell lines, some MTIs showed
significant associations with TMB levels (Figure 2A,
docetaxel: R = 0.44, p-value = 0.004) and MSI-H (Figure
2D, docetaxel: p-value < 0.001). However, no
significance was found in other tubulin related drugs,
including AURK inhibitors (Figure 2B, 2E, alisertib: p-
values > 0.05) and KIF inhibitors (Figure 2C, 2F, ARRY-
520: p-values > 0.05) (complete results were shown in
Supplementary Table 2).

Moreover, survival analysis revealed three MTIs related
genes (like BUB1B) in TMB group and 12 MTIs related
genes (like ABCG2) in MSI-H group had significant
prognostic values (Supplementary Figure 1). Together,
we referred that some MTIs were more likely to treat
GC with high immune activity.

Prediction of the mutant gene for candidate drugs
activity and its association with high immune activity
To find the characteristic gene which could suggest the
activities of MTIs in GC with high immune activity, we
started with a PPI network analysis. Based on the 17
common DEGs directly enriched in the MTIs
representative pathways, we constructed a PPI network
with 30 nodes and 289 edges (Figure 3A). After that we carried out a mutational landscape analysis on these 30 node genes and revealed that three genes (DYNC1H1, KIF26B, and CENPF) were mutated at a high frequency (mutation frequency ≥ 7%) in the TCGA-STAD cohort (Figure 3B).

To characterize the roles of these three genes in high immune activity, we separately compared the levels of TMB, MSI score, and mRNA expression between these three genes mutated and wild type tumors based on the TCGA-STAD, NCI-60 cell lines and immunotherapy datasets. As shown in Figure 4 and Supplementary Figure 2, mutations in DYNC1H1 were significantly correlated with TMB-H and MSI-H in these above datasets. Mutations in CENPF were considerably correlated with TMB-H and MSI-H in the TCGA-STAD cohort, but not in the NCI-60 cell lines or immunotherapy dataset. Mutations in KIF26B were significantly correlated with TMB-H and MSI-H in the TCGA-STAD cohort, but not immunotherapy datasets (KIF26B was not detected in NCI-60 cell lines dataset).

Besides, there was no significant difference of mRNA expression levels among three genes mutated and wild type tumors in TCGA-STAD or NCI-60 cell lines dataset (Figure 4C, 4F). Together, these results indicated that mutant DYNC1H1 was strongly associated with enhanced tumor immune activity.

Furthermore, we investigated the importance of mutant DYNC1H1 in TCGA pan-cancer, showing that the mutation frequency of DYNC1H1 was 0.4%-19.7%, average mutation frequency was 5.17% in 15 cancers. DYNC1H1 mutated tumors were associated with higher TMB levels than its wild type tumors (Supplementary Figure 3A). Accordingly, the mutation frequency of DYNC1H1 was positively associated with TMB levels in 9 cancers (Supplementary Figure 3B). Moreover, DYNC1H1 was highly mutated in Uterine Corpus Endometrial Carcinoma (UCEC) and GC. There were more than 26 missense mutation spots of DYNC1H1 in GC (Supplementary Figure 3C, 3D) These results showed that the mutant DYNC1H1 was consistent with high immune activity of TMB levels in multiple cancers.

Figure 1. Identification of candidate drugs that may treat gastric cancer with high immune activity in TCGA-STAD cohort. (A) Venn diagram (top) showing the number of common candidate drugs (CMap score < -90) from MSI-H (blue) and TMB-H (red) groups via the connectivity map database. Connectivity score table (down) displaying nine common candidate drugs, each row responsible for a drug and columns corresponding to MSI-H and TMB-H groups. The score labels representing the connectivity score of each drug in each group, and right sides of the table indicating the name and mechanism of each drug. These nine drugs can be classified into seven types. (B) GSEA results for candidate drugs based on the functional gene sets of these seven drug types from the Molecular Signatures Database by the function of enricher of clusterprofiler package. Pathways in red font were significantly enriched (p value < 0.05).
Association of mutant DYNC1H1 with enhanced microtubule inhibitors activities

To explore the interaction between mutant DYNC1H1 and MTIs, we firstly selected 41 MTIs included in NCI-60 cell lines dataset. 13 of these MTIs have significantly increased activities in the DYNC1H1 mutated cell lines (Figure 5, p-value < 0.05). Furthermore, a clear trend of higher MTIs activities was observed in DYNC1H1-mutated GC cells (Supplementary Figure 4). These results suggested that mutations in DYNC1H1 may be an essential biomarker for the antitumor effects of MTIs.

Effects of mutant DYNC1H1 on enhanced microtubule inhibitors activities

To further explore how mutant DYNC1H1 increase drug sensitivities of MTIs, we used a differential analysis method to identify mutant DYNC1H1 related genes by comparing DYNC1H1 mutated tumor with wild type tumors (37/338) in TCGA-STAD. A total of 199 genes were significantly correlated with mutant DYNC1H1 (23 up-regulated and 176 down-regulated genes) (Figure 6A). Based on NCI-60 cell lines dataset, the expressions of four genes (IGF2, MAL, KRT13, CALCA) were negatively associated with MTIs sensitivities (Figure 6B–6E, p-value < 0.05). Accordingly, IGF2 expression levels were lower in paclitaxel sensitive breast cancer cells than in paclitaxel resistant breast cancer cells in GSE90564 (Figure 6F, p-value < 0.05). Together, these results suggested that the process of mutant DYNC1H1 sensitizing MTIs may be followed by decreased expressions of MTIs related genes such as IGF2, KRT13, CALCA and MAL.

DISCUSSION

The advent of cancer immunotherapy, such as the approval of anti-PD1 monoclonal antibodies, has altered the treatment paradigm of many malignancies including GC [6, 7]. However, the clinical benefits were quite low and limited to high immune activity subtypes patients [2–5]. In this study, MTIs were repurposed as the more appropriate drugs to treat GC with high immune activity (TMB-H and MSI-H) based on CMap database and GSEA (Figure 7). Then, MTIs like docetaxel showed increased activities when the mutations in DYNC1H1.
Furthermore, mutant DYNC1H1 can act as a biomarker for MTIs activities possibly because it may be followed by lower levels of MTIs-related genes, or some mutations in DYNC1H1 may change the binding confirmation of MTIs and microtubules. In summary, our research indicated that GC patients with high immune activity may benefit from some MTIs that can be sensitized by mutant DYNC1H1.

This study presented that some MTIs may treat high immune activity subgroup GC patients by several lines of evidence. Firstly, nocodazole and albendazole were recognized as MTIs from CMap database (Figure 1A). Nocodazole can affect the dynamics of microtubules by specifically binding to the cell motility apparatus [18]. Apart from the indicator of an anthelmintic benzimidazole carbamate [19, 20], albendazole also can inhibit microtubule polymerization for the treatment of gastric cancer and other cancers [21–23]. Then, some MTIs representative pathways can be significantly enriched in high immune activity of GC: "GO_MICROTUBULE_BINDING", "GO_MICROTUBULE_DEPOLYMERIZATION", whilst other drug types repurposed by CMap showed no significance (Figure 1B). Moreover, the activities of some MTIs (such as docetaxel) were strongly higher in MSI-H and TMB-H gastric cancer cells in the GDSC database. However, activities of other tubulin related drugs (such as AURK inhibitors, KIF inhibitors) revealed no significance in different MSI status and TMB levels (Figure 2).

MTIs are widely known for binding to the microtubules [24, 25]. Among then, taxanes (paclitaxel and docetaxel) have shown antitumor activity in the treatment of GC according to the National Comprehensive Cancer Network (NCCN) guidelines. In addition to antiproliferative effects, growing studies have reported that some MTIs can be

![Figure 3. Mutational landscape of genes involved in pathways of microtubule inhibitors (MTIs) in TCGA-STAD cohort. (A) Protein-protein interaction (PPI) network of genes that significantly enriched in microtubule inhibitors (MTIs) representative gene sets; (B) Mutational landscape of node genes from PPI network showing that CENPF, KIF26B, and DYNC1H1 were highly mutated in TCGA-STAD cohort (alteration frequency ≥ 7%) by the GenVisR package. Top for somatic mutation rate of each sample, bottom left for the total mutation frequency of each gene, and bottom right for specific mutation type of each gene in each sample.](image-url)
Figure 4. Association of mutant genes with high immune activity in TCGA-STAD and NCI-60 cell lines datasets. Levels of TMB, MSI score, and mRNA expression in TCGA-STAD (A–C) and NCI-60 cell lines (D–F), stratified by genes (DYNCH1H1, CENPF, and KIF26B) mutation status. MUT: mutated, WT: wild type. KIF26B was not detected in NCI-60 cell lines dataset. All p-values were obtained by Student’s t-test, in addition to χ² test for (E). P-value < 0.05 was considered significant.

Figure 5. Association of mutant DYNCH1H1 with enhanced microtubule inhibitors (MTIs) activities in NCI-60 cell lines dataset. (A) Volcano plot for the different activities of MTIs between DYNCH1H1 mutated and wild type NCI-60 cell lines dataset. The x-axis represented the different levels of mean -logGI50, and the y-axis showed p-values obtained by Student’s t-test. (B–E) Histograms depicting different MTIs activities of nocodazole (B), docetaxel (C), colchicine (D), vincristine (E), stratified by DYNCH1H1 mutation status in NCI-60 cell lines dataset (p-value < 0.05 by Student’s t-test). MUT: mutated, WT: wild type. P-value <0.05 was considered significant. DYNCH1H1 was mutated in six NCI-60 cell lines: HCC_2998, HCT_116, HCT_15, KM12, MOLT_4, and UACC_62.
immunostimulatory properties, including their abilities to reprogram the immune-suppressive M2 profile of tumor-associated macrophages to immune-stimulating M1 profile [26], stimulate maturation and activities of dendritic cells [27, 28], and decrease the accumulation and immunosuppressive activities of tumor-infiltrating MDSCs [29]. Clinically, single-agent paclitaxel even showed a better median progression-free survival than the anti-PD1 pembrolizumab (4.1 months vs 1.5 months) in the KEYNOTE-061 clinical trial (PD1 CPS>1) [4]. These evidences supported that some MTIs may achieve good outcomes in gastric cancer patients with high immune activity.

Our results clearly demonstrated that drug activities of some MTIs were strongly associated with mutant DYNC1H1 (Figure 6). DYNC1H1 is the heavy chain of cytoplasmic dynein which acts as a motor protein using ATP to travel along the microtubule (MTs) toward minus end [30, 31]. This complex participates in multiple cell processes, such as spindle formation in mitosis and transportation of various cellular cargos. Mutant DYNC1H1 was reported to impede the ATP hydrolysis cycle which help bind to MTs in the neurological diseases [32]. Most mutations of DYNC1H1 were tied up with the occurrence and development of pancreatic cancer [33–35], suggesting that mutations in DYNC1H1 may play a vital role in the complex biological process of malignant tumors. However, the effect of its mutation on gastric cancer remains unknown.

In this study, mutant DYNC1H1 was closely associated with MTIs-related genes (IGF2, KRT13, MAL, and CALCA) (Figure 5). IGF2 and anti-apoptotic gene KRT1 were significantly overexpressed in MTI-resistant cancer cells, and exhaustion of IGF2 can restore paclitaxel sensitivity [36, 37]. Also, CALCA and MAL were reported to be highly expressed in the allergic reactions of MTIs [38, 39]. On the other hand, DYNC1H1 directly binds to MTs via the structure called MTBD [40, 30]. Besides, MTIs (like paclitaxel

![Figure 6. Effects of mutant DYNC1H1 on enhanced microtubule inhibitors (MTIs) activities.](image-url)

(A) Volcano plot for the DEGs between DYNC1H1 mutated and wild type gastric cancer patients in TCGA-STAD cohort. The x-axis represented log2 (fold change): mutant DYNC1H1 compared with wild type patients, and the y-axis represented significant difference as −log10(FDR). The criteria of FDR <0.05 and |log2FC| ≥ 2 were considered significant by the function TCGAbiolinks_DEA of TCGAbiolinks. (B–E) Correlations between MTIs activities and mRNA expressions of IGF2, KRT13, CALCA, and MAL in NCI-60 cell lines dataset, respectively. P-value estimated using Pearson’s correlation. The x-axis represented the correlation coefficient, and the y-axis showed the significance (−log10 p-value). (F) Histograms depicting different mRNA expressions of IGF2 between MTIs sensitive and resistant cell lines in GSE90564 dataset. P-value estimated using Student’s t-test. P-value <0.05 was considered significant.
and docetaxel) exert antitumor effects through binding to β-tubulin [41, 42]. Hence we speculated that mutant DYNC1H1 may narrow the binding pocket of MTBD and β-tubulin and then increase binding pocket of MTIs and β-tubulin, thereby enhancing the anti-microtubule effect of MTIs. We identified 26 missense mutation sites of DYNC1H1 in the TCGA-STAD mutation profile (Supplementary Figure 3). However, there were no studies clearly illustrated the correlation of these mutation sites of DYNC1H1 with gastric cancer.

Our study has some limitations such as few normal samples included in this study but no other suitable datasets available for validation. We cannot also rule out the possibility that other drugs or combination therapy may show more benefits for GC patients with high immune activity. Therefore, we plan to use our own clinical samples for verification analysis in the future. Further investigation of how mutant DYNC1H1 sensitizes MTIs will be necessary in future studies.

To conclude, our study identified some MTIs such as docetaxel that could potentially be the best drugs for GC with high immune activity (TMB-H and MSI-H). Mutant DYNC1H1 significantly positively correlated with TMB-H and MSI-H in GC or various cancers. We found that mutant DYNC1H1 can sensitize MTIs, possibly because it was accompanied with down-regulation of some MTIs resistant or side effect genes. It may also change the binding pocket of MTIs and microtubules.

Figure 7. Work flow of the study. DEGs: differentially expressed genes; TMB-H: high tumor mutational burden; MSI-H: high microsatellite instability; GSEA: gene sets enrichment analysis; GDSC: genomics of drug sensitivity in cancer; PPI: protein-protein interaction; TCGA: The Cancer Genome Atlas; NCI-60: National Cancer Institute 60.
**MATERIALS AND METHODS**

Gastric cancer dataset and differentially expressed genes (DEGs) associated with high immune activity

GC dataset was acquired from GDC Data Portal TCGA-STAD (n = 407). "Level 3" RNA sequencing data (raw count) and clinical information were downloaded by using R package TCGAbiolinks [43]. Then, patients were classified into two groups: TMB-H group (median as cut-point of tumor mutational rate), and MSI-H group. Finally, differential analysis of the two groups were both used by the function TCGAbiolinks_DEA. DEGs were determined with the criteria of $|\log_2(FC)| > 2$ and FDR $< 0.05$.

**Drug prediction**

The CMap database ([https://clue.io/cmap](https://clue.io/cmap)) was used to identify drugs that can effectively treat high immune activity subgroups GC. Input data require a range of 10 ~ 150 genes that are up-regulated and/or down-regulated. Because the number of DEGs in the two groups of TMB-H and MSI-H were more than 150, we selected top150 up-regulated DEGs following the FDR in ascending order. A so-called Connectivity Score (-100 ~100) is an indicator for evaluating the correlation between a drug and input genes. The score less than -90 can be considered that the drug is significantly negatively related to the input genes, that is, the drug can dramatically reverse the role of these genes to treat specific diseases. Drugs correlated with the input genes of TMB-H and MSI-H group were selected as candidate drugs for subsequent analysis.

**Gene sets enrichment analysis (GSEA)**

GSEA was used to further screen the significant candidate drugs. Candidate drugs-related gene sets were selected from MSigDB [44], and common DEGs were used as input data. GSEA was performed separately with gene sets of each drug type using the function enricher of clusterprofiler [45] package. The drug corresponding to the significantly enriched gene sets ($p$-value $\leq 0.05$) was regarded as significant candidate drug.

**Gastric cancer data of drug sensitivity and high immune activity**

To validate the role of significant candidate drugs for treating gastric cancer with high immune activity, we extracted the half maximal inhibitory concentration (IC50) values of them in GC cell lines from GDSC ([https://www.cancerrxgene.org/downloads/anova](https://www.cancerrxgene.org/downloads/anova)) database. MSI status of each GC cell line was also provided by GDSC. TMB levels of each cell line were retrieved from the CCLE ([https://portals.broadinstitute.org/ccle/data](https://portals.broadinstitute.org/ccle/data)) database.

**Protein-protein interaction (PPI) network**

Search Tool for the Retrieval of Interacting Genes (STRING; string-db.org) was used to build a PPI network for genes directly enriched in gene sets of significant candidate drugs. The parameter of interaction was set as the interaction score $> 0.9$ and no more than 20 interactors. Cytoscape software 3.7.0 was used to visualize the PPI network.

**Mutational landscape analysis**

Somatic mutational landscape analysis was used to explore characteristic genes of significant candidate drugs. Mutational profile of TCGA-STAD was obtained from TCGA data portal by the GDCquery_Maf function of the TCGAbiolinks package in R. Using "GenVisR" package to process and visualize the mutational burden of PPI network node genes. The specific mutant genes were identified with mutation frequency $\geq 7\%$ [46].

**Data of gene mutation and high immune activity**

To compare different levels of high immune activity (TMB, MSI) and mRNA expression between specific mutant genes mutated and wild type tumors. TCGA-STAD, NCI-60, immunotherapy cohort (Allen cohort), and TCGA pan-cancer datasets were selected.

For TCGA-STAD dataset, gene mutational profile and mRNA expression data, TMB levels (tumor mutational rate), MSI score [47] data were used. For NCI-60 cancer cell lines dataset, binary gene mutation and mRNA expression data were available from R package recellminer [14], TMB levels of each cell line were downloaded from cbioportal ([https://www.cbioportal.org](https://www.cbioportal.org)) database, and microsatellite status data were downloaded from cosmic ([https://cancer.sanger.ac.uk/cosmic#](https://cancer.sanger.ac.uk/cosmic#)) database. For Allen cohort, consisting of 110 advanced-stage melanoma patients treated with anti-CTLA-4 therapy, provided complete somatic mutation profile [48]. For TCGA pan-cancer, mutational profiles were obtained from the TCGA data portal by the GDCquery_Maf function of the TCGAbiolinks package in R. TMB levels were calculated based on non-synonymous data. Mutation frequencies of specific mutant genes in each cancer type were estimated respectively.

**Data of drug sensitivity and gene mutation**

To verify the relationship between specific mutant genes and significant candidate drugs, we used
genomics data and drug sensitivity of NCI-60 cell lines dataset and GDSC database. For NCI-60 cell lines, the concentration of drug was presented as cause 50% growth inhibition (GI50) value and was available from R package rcellminer. Gene mutation information was described above Gastric cancer data of drug sensitivity and high immune activity. For GDSC database, gene mutation information was downloaded from the GDSC website (https://www.cancerrxgene.org/downloads). IC50 values of candidate drugs were described above. Data of gene mutation and high immune activity.

The effect of specific mutant genes

To investigate the impact of specific mutant genes on significant candidate drugs. Firstly, we used differential analysis method to find genes significantly associated with the specific mutant genes. According to significant mutant genes status, DEGs were identified by the mutated group compared to the wild type group in the TCGA-STAD (the criteria of |log2 (FC)|> 2 and FDR <0.05). Secondly, to analyze the relationships between these DEGs and significant candidate drugs activities, the gene expression data and drug activity data of NCI-60 cell lines and GSE90564 (paclitaxel sensitivity and resistance dataset, n = 38) were used.

Statistical analysis

Differential analysis was mainly conducted by the function TCGAbiolinks_DEA of TCGAbiolinks package. Survival curves were performed by the Kaplan-Meier method and compared by the log-rank test via the R package survival. Group comparisons were performed by Student’s t test for continuous variables, while χ² test for categorical variables. The correlation of IC50 values of candidate drugs with TMB levels in GC cell lines of GDSC database, GI50 values of candidate drugs with gene expression or gene mutation in NCI-60 cancer cell lines dataset were identified by Spearman’s correlation analysis. All statistical tests were two-sided, and p-value <0.05 was considered significant. Statistical analysis was performed by R software version 3.6.1 (v. 3.5.2 http://www.r-project.org).

Abbreviations

ICB: immune checkpoint blockade; GC: gastric cancer; STAD: stomach adenocarcinoma; TCGA: The Cancer Genome Atlas; MSI-H: high microsatellite instability; TMB-H: high tumor mutational burden; CMap: Connectivity Map; MTIs: microtubule inhibitors; DYNC1H1: Dynein Cytoplasmic 1 Heavy Chain 1; DEGs: differentially expressed genes; GSEA: gene sets enrichment analysis; PPI: protein-protein interaction; NCI-60: National Cancer Institute 60; GDSC: genomics of drug sensitivity in cancer; IC50: inhibitory concentration; GI50: growth inhibition; UCEC: Uterine Corpus Endometrial Carcinoma.

AUTHOR CONTRIBUTIONS

Zhi Li conceived the study. Jin Bai performed all bioinformatic analyses and edited the manuscript. BoWen Yang, Ruichuan Shi, Xinye Shao provided assistance in mutational landscape analysis, data collection, and statistical analysis, respectively. Yujing Yang and Fang Wang revised the manuscript. Xiujuan Qu, Yunpeng Liu, Jiawen Xiao, and Ye Zhang discussed the results and provided guidance to the study. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, Ministry of Education (China Medical University, Shenyang, China) for providing the space and equipment for conducting the experiments.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

FUNDING

The National Key Research and Development Program of China (NO.2017YFC1308900); National Natural Science Foundation of China (NO.81972331, No. 81302023); The General Projects of Liaoning Province Colleges and Universities (LFWK201706).

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–74. https://doi.org/10.1016/j.cell.2011.02.013 PMID:21376230
2. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, Sun W, Jalal SI, Shah MA, Metges JP, Garrido M, Golan T, Mandala M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. JAMA Oncol. 2018; 4:e180013. https://doi.org/10.1001/jamaoncol.2018.0013 PMID:29543932
3. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, et al. Comprehensive molecular
characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med. 2018; 24:1449–58. https://doi.org/10.1038/s41591-018-0101-z PMID:30013197

4. Shibata K, Özgüroğlu M, Bang YJ, Di Bartolomeo M, Mandalà M, Ryu MH, Fornaro L, Olesinski T, Caglicevic C, Chung HN, Muro K, Goekkurt E, Mansoor W, et al, and KEYNOTE-061 investigators. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. Lancet. 2018; 392:123–33. https://doi.org/10.1016/S0140-6736(18)31257-1 PMID:29880231

5. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, Yuan XL, Chen Y, Yang SJ, Shi JH, Hu XC, Lin XY, Zhang QY, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemoresistant gastric cancer treated with toripalimab, a PD-1 antibody in phase I/II clinical trial NCT02915432. Ann Oncol. 2019; 30:1479–86. https://doi.org/10.1093/annonc/mdz197 PMID:31236579

6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012; 366:2443–54. https://doi.org/10.1056/NEJMoa1200690 PMID:22658127

7. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012; 366:2455–65. https://doi.org/10.1056/NEJMoa1200694 PMID:22658128

8. Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, Yeh KH, Yoshikawa T, Oh SC, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017; 390:2461–2471. https://doi.org/10.1016/S0140-6736(17)31827-5 PMID:28993052

9. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol. 2016; 17:717–26. https://doi.org/10.1016/S1470-2241(16)00175-3 PMID:27157491

10. Salati M, Orsi G, Smyth E, Aprile G, Beretta G, De Vita F, Di Bartolomeo M, Fanotto V, Lonardi S, Morano F, Pietrantonio F, Pinto C, Rimassa L, et al. Gastric cancer: translating novels concepts into clinical practice. Cancer Treat Rev. 2019; 79:101889. https://doi.org/10.1016/j.ctrv.2019.101889 PMID:31445415

11. Boku N, Ryu MH, Kato K, Chung HC, Minashi K, Lee KW, Cho H, Kang WK, Komatsu Y, Tsuda M, Yamaguchi K, Hara H, Fumita S, et al. Safety and efficacy of nivolumab in combination with S1-capecitabine plus oxaliplatin in patients with previously untreated, unresectable, advanced, or recurrent gastric/ gastroesophageal junction cancer: interim results of a randomized, phase II trial (ATTRACTION-4). Ann Oncol. 2019; 30:250–58. https://doi.org/10.1093/annonc/mdy540 PMID:30566590

12. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, Lahr DL, Hirschman JE, Liu Z, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell. 2017; 171:1437–52.e17. https://doi.org/10.1016/j.cell.2017.10.049 PMID:29195078

13. Gong C, Hu Y, Zhou M, Yao M, Ning Z, Wang Z, Ren J. Identification of specific modules and hub genes associated with the progression of gastric cancer. Carcinogenesis. 2019; 40:1269–77. https://doi.org/10.1093/carcin/bgz040 PMID:30805585

14. Reinhold WC, Sunshine M, Liu H, Varma S, Kohn KW, Morris J, Doroshow J, Pommier Y. CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. Cancer Res. 2012; 72:3499–511. https://doi.org/10.1158/0008-5472.CAN-12-1370 PMID:22802077

15. Baba H, Watanabe M, Okabe H, Miyamoto Y, Sakamoto Y, Baba Y, Iwatsuki M, Chikamoto A, Beppu T. Upregulation of ERCC1 and DPD expressions after oxaliplatin-based first-line chemotherapy for metastatic colorectal cancer. Br J Cancer. 2012; 107:1950–55. https://doi.org/10.1038/bjc.2012.502 PMID:23169295

16. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, Bindal N, Beare D, Smith JA, Thompson IR,
Ramawaty S, Futreal PA, Haber DA, et al. Genomics of drug sensitivity in cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res. 2013; 41:D955–61. https://doi.org/10.1093/nar/gks1111 PMID: 23180760

17. Lee JH, Park YR, Jung M, Lim SG. Gene regulatory network analysis with drug sensitivity reveals synergistic effects of combinatorial chemotherapy in gastric cancer. Sci Rep. 2020; 10:3932. https://doi.org/10.1038/s41598-020-61016-z PMID: 32127608

18. Florian S, Mitchison TJ. Anti-microtubule drugs. Methods Mol Biol. 2016; 1413:40–21. https://doi.org/10.1007/978-1-4939-3542-0_25 PMID: 27193863

19. Köhler P. The biochemical basis of anthelminthic action and resistance. Int J Parasitol. 2001; 31:336–45. https://doi.org/10.1016/s0020-7519(01)00131-x PMID: 11400692

20. Gil-Grande LA, Rodriguez-Caabeiro F, Prieto JG, Sánchez-Ruano JJ, Brasa C, Aguilar L, García-Hoz F, Casado N, Bárcena R, Alvarez AI, Dal-Ré R. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. Lancet. 1993; 342:1269–72. https://doi.org/10.1016/0140-6736(93)92361-v PMID: 7901585

21. Zhang X, Zhao J, Gao X, Pei D, Gao C. Anthelminthic drug albendazole arrests human gastric cancer cells at the mitotic phase and induces apoptosis. Exp Ther Med. 2017; 13:595–603. https://doi.org/10.3892/etm.2016.3992 PMID: 28352336

22. Pourgholami MH, Akhter J, Wang L, Lu Y, Morris DL. Antitumor activity of albendazole against the human colorectal cancer cell line HT-29: in vitro and in a xenograft model of peritoneal carcinomatosis. Cancer Chemother Pharmacol. 2005; 55:425–32. https://doi.org/10.1007/s00280-004-0927-6 PMID: 15565325

23. Patel K, Doudican NA, Schiff PB, Orlow SJ. Albendazole sensitizes cancer cells to ionizing radiation. Radiat Oncol. 2011; 6:160. https://doi.org/10.1186/1748-717X-6-160 PMID: 22094106

24. Field JJ, Kanakankantha A, Miller JH. Microtubule-targeting agents are clinically successful due to both mitotic and interphase impairment of microtubule function. Bioorg Med Chem. 2014; 22:5050–59. https://doi.org/10.1016/j.bmc.2014.02.035 PMID: 24650703

25. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov. 2010; 9:790–803. https://doi.org/10.1038/nrd3253 PMID: 20885410

26. Wanderley CW, Colón DF, Luiz JP, Oliveira FF, Viacava PR, Leite CA, Pereira JA, Silva CM, Silva CR, Silva RL, Speck-Hernandez CA, Mota JM, Alves-Filho JC, et al. Paclitaxel reduces tumor growth by reprogramming tumor-associated macrophages to an M1 profile in a TLR4-dependent manner. Cancer Res. 2018; 78:5891–900. https://doi.org/10.1158/0008-5472.CAN-17-3480 PMID: 30104241

27. Shurin GV, Tourkova IL, Kaneno R, Shurin MR. Chemotherapeutic agents in noncytotoxic concentrations increase antigen presentation by dendritic cells via an IL-12-dependent mechanism. J Immunol. 2009; 183:137–44. https://doi.org/10.4049/jimmunol.0900734 PMID: 19535620

28. Kaneno R, Shurin GV, Tourkova IL, Shurin MR. Chemomodulation of human dendritic cell function by antineoplastic agents in low noncytotoxic concentrations. J Transl Med. 2009; 7:58. https://doi.org/10.1186/1479-5876-7-58 PMID: 19591684

29. Sevko A, Michels T, Vrohlings M, Umansky L, Beckhove P, Kato M, Shurin GV, Shurin MR, Umansky V. Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. J Immunol. 2013; 190:2464–71. https://doi.org/10.4049/jimmunol.1202781 PMID: 23359505

30. Nishida N, Komori Y, Takarada O, Watanabe A, Tamura S, Kubo S, Shimada I, Kikkawa M. Structural basis for two-way communication between dynesin and microtubules. Nat Commun. 2020; 11:1038. https://doi.org/10.1038/s41467-020-14842-8 PMID: 32098965

31. Cianfrocco MA, DeSantis ME, Leschziner AE, Reck-Peterson SL. Mechanism and regulation of cytoplasmic dynein. Annu Rev Cell Dev Biol. 2015; 31:83–108. https://doi.org/10.1146/annurev-cellbio-100814-125438 PMID: 26436706

32. Hoang HT, Schlager MA, Carter AP, Bullock SL. DYNC1H1 mutations associated with neurological diseases compromise processivity of dynein-dynactin-cargo adaptor complexes. Proc Natl Acad Sci USA. 2017; 114:E1597–606. https://doi.org/10.1073/pnas.1620141114 PMID: 28196890
33. Gong LB, Wen T, Li Z, Xin X, Che XF, Wang J, Liu YP, Qu XJ. DYNC1I1 promotes the proliferation and migration of gastric cancer by up-regulating IL-6 expression. Front Oncol. 2019; 9:491. https://doi.org/10.3389/fonc.2019.00491 PMID: 31249807

34. Palaniappan A, Ramar K, Ramalingam S. Computational identification of novel stage-specific biomarkers in colorectal cancer progression. PLoS One. 2016; 11:e0156665. https://doi.org/10.1371/journal.pone.0156665 PMID: 27243824

35. Sucularli C, Arslantas M. Computational prediction and analysis of deleterious cancer associated missense mutations in DYNC1H1. Mol Cell Probes. 2017; 34:21–29. https://doi.org/10.1016/j.mcp.2017.04.004 PMID: 28455235

36. Huang GS, Brouwer-Visser J, Ramirez MJ, Kim CH, Hebert TM, Lin J, Arias-Pulido H, Qualls CR, Prossnitz ER, Goldberg GL, Smith HO, Horwitz SB. Insulin-like growth factor 2 expression modulates taxol resistance and is a candidate biomarker for reduced disease-free survival in ovarian cancer. Clin Cancer Res. 2010; 16:2999–3010. https://doi.org/10.1158/1078-0432.CCR-09-3233 PMID: 20404007

37. Lian F, Xing X, Yuan G, Schäfer C, Rauser S, Walch A, Röcken C, Ebeling M, Wright MB, Schmid RM, Ebert MP, Burgermeister E. Farnesoid X receptor protects human and murine gastric epithelial cells against inflammation-induced damage. Biochem J. 2011; 438:315–23. https://doi.org/10.1042/BJ20102096 PMID: 21619550

38. Itoh Y, Sendo T, Hirakawa T, Takasaki S, Goromaru T, Nakano H, Oishi R. Pemirolast potently attenuates paclitaxel hypersensitivity reactions through inhibition of the release of sensory neuropeptides in rats. Neuropharmacology. 2004; 46:888–94. https://doi.org/10.1016/j.neuropharm.2003.11.018 PMID: 15033348

39. Roglio I, Bianchi R, Camozzi F, Carozzi V, Cervellini I, Crippa D, Lauria G, Cavaletti G, Melcangi RC. Docetaxel-induced peripheral neuropathy: protective effects of dihydroprogesterone and progesterone in an experimental model. J Peripher Nerv Syst. 2009; 14:36–44. https://doi.org/10.1111/j.1529-8027.2009.00204.x PMID: 19335538

40. Kon T, Sutoh K, Kurisu G. X-ray structure of a functional full-length dynein motor domain. Nat Struct Mol Biol. 2011; 18:638–42. https://doi.org/10.1038/nsmb.2074 PMID: 21602819

41. Schiff PB, Horwitz SB. Taxol assembles tubulin in the absence of exogenous guanosine 5′-triphosphate or microtubule-associated proteins. Biochemistry. 1981; 20:3247–52. https://doi.org/10.1021/bi00514a041 PMID: 6113842

42. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by taxol. Nature. 1979; 277:665–67. https://doi.org/10.1038/277665a0 PMID: 423966

43. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sacedot TS, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G, Noushmehr H. TCGAbiolinks: an r/bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res. 2016; 44:e71. https://doi.org/10.1093/nar/gkv1507 PMID: 26704973

44. Li Z, Li AD, Xu L, Bai DW, Hou KZ, Zheng HC, Qu XJ, Liu YP. SPARC expression in gastric cancer predicts poor prognosis: results from a clinical cohort, pooled analysis and GSEA assay. Oncotarget. 2016; 7:70211–22. https://doi.org/10.18632/oncotarget.12191 PMID: 28053291

45. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012; 16:284–87. https://doi.org/10.1089/omi.2011.0118 PMID: 22455463

46. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, Castro MA, Gibb EA, Kanchi RS, et al, and TCGA Research Network. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2017; 171:540–56.e25. https://doi.org/10.1016/j.cell.2017.09.007 PMID: 28988769

47. Bonneville R, Krouk MA, Kautto EA, Miyazawa J, Wing MR, Chen HZ, Reeser JW, Yu L, Roychowdhury S. Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol. 2017; 2017:PO.17.00073. https://doi.org/10.1200/PO.17.00073 PMID: 29850653

48. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Foppen MH, Goldinger SM, Utikal J, Hassel JC, Weide B, et al. Genomic
correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015; 350:207–11.
https://doi.org/10.1126/science.aad0095
PMID:26359337
SUPPLEMENTARY MATERIALS

Supplementary Figures

Supplementary Figure 1. The microtubule inhibitors (MTIs) related DEGs in the survival analysis of TCGA-STAD cohort. Kaplan-Meier plots for overall survival of DEGs related to MTIs in TMB-H group (A) and MSI-H group (B) by log-rank test. P-value <0.05 was considered significant.
Supplementary Figure 2. Association of mutant DYNC1H1 with TMB in immunotherapy cohort. Levels of TMB in immunotherapy cohort (Allen cohort), stratified by genes (DYNC1H1, KIF26B, and CENPF) mutation status. MUT: mutated, WT: wild type. Student's t-test obtained p-values. ( * P < 0.05; ns, not significant).

Supplementary Figure 3. Association of mutant DYNC1H1 with TMB in various cancers. (A) Line chart showing DYNC1H1 mutation frequency (green) and levels of TMB with DYNC1H1 mutated (red) and wild type (blue) in 15 cancers. (B) Line chart presenting DYNC1H1 mutation frequency (green) and levels of TMB (red) in 9 cancers. (C, D) Lollipop plots presenting the mutation spots of DYNC1H1 in gastric cancer (GC)(C) and Uterine Corpus Endometrial Carcinoma (UCEC)(D). Abbreviation: UCEC, Uterine Corpus Endometrial Carcinoma; STAD, Stomach Adenocarcinoma; SKCM, Skin Cutaneous Melanoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; UCS, Uterine Carcinosarcoma; LUAD, Lung Adenocarcinoma; OV, Ovarian serous cystadenocarcinoma; HNSC, Head and Neck Cancer; ESCA, Esophageal Cancer; LIHC, Liver hepatocellular carcinoma; BRCA, Breast invasive carcinoma; KIRP, Kidney renal papillary cell carcinoma; KIRC, Kidney renal clear cell carcinoma.
Supplementary Figure 4. Association of mutant DYNC1H1 with microtubule inhibitors (MTIs) in gastric cancer cell lines with high immune activity based on GDSC database. Histograms depicting different MTIs activities of docetaxel and vinorelbine, stratified by DYNC1H1 status in gastric cancer cell lines based on GDSC database (p-value < 0.05 by Student’s t-test). MUT: mutated, WT: wild type.
SUPPLEMENTARY TABLES

Please browse Full Text version to see the data of Supplementary Tables 1 and 3.

Supplementary Table 1. The common DEGs of MSI-H group and TMB-H group.

Supplementary Table 2. The functional gene sets of each drug type from molecular signatures database.

| Candidate drug types | Functional gene sets |
|----------------------|----------------------|
| 1. Microtubule inhibitor | BIOCARTA_G2_PATHWAY  
BIOCARTA_MTOR_PATHWAY  
BIOCARTA_NFKB_PATHWAY  
GO_MICROTUBULE  
GO_MICROTUBULE_BINDING  
GO_MICROTUBULE_DEPOLYMERIZATION  
GO_MICROTUBULE_NUCLEATION  
GO_MICROTUBULE_POLYMERIZATION  
GO_MICROTUBULE_POLYMERIZATION_OR_DEPOLYMERIZATION  
GO_REGULATION_OF_MICROTUBULE_POLYMERIZATION_OR_DEPOLYMERIZATION  
GO_S_ADENOSYLHOMOCYSTEINE_METABOLIC_PROCESS  
MICROTUBULE  
MICROTUBULE_BINDING  
MICROTUBULE_POLYMERIZATION_OR_DEPOLYMERIZATION  
REACTOME_POST_CHAPERONIN_TUBULIN_FOLDING_PATHWAY  
REACTOME_FORMATION_OF_TUBULIN_FOLDING_INTERMEDIATES_BY_CCT_TRIC |
| 2. Corticosteroid agonist | GO_CELLULAR_RESPONSE_TO_CORTICOSTEROID_STIMULUS  
GO_REGULATION_OF_CORTICOSTEROID_HORMONE_SECRETION  
GO_RESPONSE_TO_CORTICOSTEROID  
GO_RESPONSE_TO_CORTICOSTERONE |
| 3. Androgen receptor agonist | GO_ANDROGEN_RECEPTOR_BINDING  
GO_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY  
GO_NEGATIVE_REGULATION_OF_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY  
GO_REGULATION_OF_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY  
GO_TESTOSTERONE_BIOSYNTHETIC_PROCESS |
| 4. Protein synthesis inhibitor | GO_POLYSOME  
GO_RIBOSOMAL_SMALL_SUBUNIT_BIOGENESIS  
GO_RIBOSOMAL_LARGE_SUBUNIT_BIOGENESIS  
GO_RIBOSOME_BIOGENESIS  
GO_SIGNAL_RECOGNITION_PARTICLE_ENDOPLASMIC_RETICULUM_TARGETING  
GO_TRNA_CATABOLIC_PROCESS  
GO_TRNA_METABOLIC_PROCESS  
GO_EUKARYOTIC_TRANSLATION_INITIATION_FACTOR_3_COMPLEX |
| 5. Cyclooxygenase inhibitor | 0 |
| 6. Leukotriene receptor antagonist | GO_LEUKOTRIENE_D4_BIOSYNTHETIC_PROCESS |
Supplementary Table 3. Association of tubulin related drugs in gastric cancer cell lines with high immune activity based on GDSC database.

7. Adrenergic receptor antagonist

| Gene Ontology Term | Description |
|--------------------|-------------|
| GO_ALPHA_2A_ADRENERGIC_RECEPTOR_BINDING | Adrenergic receptor binding |
| GO_ADRENERGIC_RECEPTOR_BINDING | Adrenergic receptor binding |
| GO_ADRENERGIC_RECEPTOR_SIGNALING_PATHWAY | Adrenergic receptor signaling pathway |
| GO_BETA_2_ADRENERGIC_RECEPTOR_BINDING | Beta 2 adrenergic receptor binding |
| GO_NEGATIVE_REGULATION_OF_ADENYLATE_CYCLASE_ACTIVATING_ADRENERGIC_RECEPTOR_SIGNALING_PATHWAY | Negative regulation of adenylate cyclase activating adrenergic receptor signaling pathway |