Original Article

Chemosensitivity of gastric cancer: analysis of key pathogenic transcription factors

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Background: We aimed to screen the key pathogenic transcription factors of gastric cancer and analyzed the correlation between the expression of transcription factors and chemotherapy drugs in gastric cancer.

Methods: Gastric cancer RNA sequencing data sets, single nucleotide polymorphism data sets, and corresponding clinical data sets were downloaded from The Cancer Genome Atlas, which is public data. The expression of transcription factors in gastric cancer and normal tissues was analyzed with R software. Pathway enrichment analysis of differentially expressed transcription factors was performed using the Kyoto Encyclopedia of Genes and Genomes database. Univariate Cox analysis was used to explore the correlation between the differential expression of transcription factors and prognosis. The interaction network among differentially expressed transcription factors was constructed using String database. Spearman test was used to explore the correlation between transcription factor mutation and gene expression. The Genomics of Drug Sensitivity in Cancer database was used to examine the relationship between the expression of transcription factors and chemotherapeutic drug sensitivity.

Results: A total of 17 differentially expressed transcription factors were screened. The results indicated that *CENPA*, *E2F1*, *EMX1*, *HOXA9*, *FOXM1*, and *MYBL2* were prognostic risk factors for gastric cancer patients ($P<0.05$), while *RXRG* and *SOX4* were prognostic protective factors for gastric cancer patients ($P<0.05$). *FDXM1* interacted with *E2F7*, *MYBL2*, *E2F1*, *NCAPG*, and *SOX9*. *FOXM1* gene mutation was positively correlated with the expression level ($P<0.05$). Based on the median value of *FOXM1*, the patients were divided into high expression group and low expression group of *FOXM1*. There was no significant difference in IC50 of 5-fluorouracil between the *FOXM1* high expression group and the *FOXM1* low expression group ($P>0.05$). The IC50 of paclitaxel in the *FOXM1* high expression group was higher than that in the *FOXM1* low expression group ($P<0.001$). The expression of IC50 of cisplatin in the *FOXM1* high expression group was higher than that in the *FOXM1* low expression group ($P<0.05$).

Conclusions: *FOXM1* was a highly expressed transcription factor in gastric cancer. High *FOXM1* expression was associated with the resistance of gastric cancer patients to paclitaxel and cisplatin. Therefore, *FOXM1* is a potential therapeutic target for gastric cancer.

Keywords: Gastric cancer; chemotherapy; transcription factors

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Introduction

Gastric cancer is one of the most common malignant tumors globally and the second leading cause of cancer death (1). At the time of diagnosis, about 30% of patients with gastric cancer have progressed to an advanced stage (2). Chemotherapy is one of the standard treatments for most advanced gastric cancers. However, due to tumor heterogeneity and individual differences, there are significant differences in the sensitivity of different patients to chemotherapy drugs (3). Multidrug resistance of patients to chemotherapy drugs is the main reason that treatment of gastric cancer fails (4). The effect of chemotherapy in patients with gastric cancer depends on chemotherapeutic drugs and is directly or indirectly affected by function-related genes (5). Individual gene changes, or polymorphisms, significantly affect patients' responses to specific chemotherapy drugs. Therefore, exploring the key pathogenic genes of gastric cancer and their mechanism of action can provide the basis for clinical chemotherapeutic drug selection, promote the development of individualized treatment, and improve the prognosis of gastric cancer patients.

The Genomics of Drug Sensitivity in Cancer (GDSC) database is a collection of data from 75,000 experiments describing the response of approximately 200 anticancer drugs in more than 1,000 tumor cells. The GDSC database can be used to analyze the sensitivity and response of tumor cells to drugs. Variations in the cancer genome can affect the efficacy of clinical treatments, and different targets respond to drugs differently. GDSC data are important for discovering potential tumor therapeutic targets. A study (6) used GDSC database analysis to determine the characteristic gene sets of advanced gastric cancer and the correlation between these gene sets and fluorouracil, which can provide clues for determining the best combination of chemotherapy drugs for gastric cancer patients. Another study (7) constructed a risk score based on gene expression, which was used to predict the survival of gastric cancer patients and the sensitivity to chemotherapy drugs.

In this study, the differentially expressed transcription factors in gastric cancer and normal gastric tissues were screened using the RNA sequencing (RNA-seq) data of gastric cancer in the Cancer Genome Atlas (TCGA) database. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, interaction network construction, and prognostic correlation analysis were used to analyze the differentially expressed transcription factors. The interaction network and prognostic correlation analysis determined *FOXM1* as a critical gene. We used GDSC to explore the correlation between *FOXM1* gene expression and gene mutation and chemosensitivity. We present the following article in accordance with the STREGA reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-274/rc).

Methods

Data download

RNA-seq data of gastric cancer were downloaded from TCGA, which is public data (normal tissue =32 cases, gastric cancer tissue =375 cases). The downloaded RNA-seq data were combined into a gene expression matrix, and the matrix was normalized and logarithmically transformed. Tumor-related transcription factors were obtained according to Cistrome Project, and their expression was extracted from the data matrix to obtain a new matrix. The row name of the matrix was set as the sample serial number, and the column name was set as the expression of transcription factors. Single nucleotide polymorphism (SNP) data were downloaded from the TCGA database to obtain the mutation status of transcription factors in each sample. The corresponding clinical data of gastric cancer patients were downloaded from the TCGA database, including patient age, gender, survival status, and survival time. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differential expression analysis of transcription factors

The differential expression of transcription factors between gastric cancer and normal tissues was analyzed with the edgeR package. Fold change (FC) was the ratio of expression in gastric cancer tissue to that in normal tissue. In this study, the transcription factors differentially expressed in normal tissues and gastric cancer were screened under the screening conditions of $|\log2FC| \geq 1$ and false discovery rate (FDR) <0.05.

Pathway enrichment analysis of KEGG

The KEGG database was used to analyze the pathway enrichment of differentially expressed transcription factors. FDR <0.05 was used as the screening standard to screen the pathways with significant differences.

Table 1 Differential expression of transcription factors between normal gastric tissue and gastric cancer tissue

Gene	Normal	Tumor	Log2FC	FDR
RXRG	0.97	0.14	−2.85	1.38E-11
MYH11	622.04	117.87	−2.40	1.55E-07
SOX9	17.15	69.19	2.01	1.07E-13
CENPA	1.28	5.18	2.02	1.50E-12
NCAPG	1.22	5.07	2.06	2.00E-12
SOX4	8.33	35.63	2.10	8.69E-14
MYB	1.35	5.79	2.10	1.78E-08
E2F1	2.58	11.11	2.11	1.61E-13
FOXM1	3.59	16.97	2.24	2.46E-13
MYBL2	7.70	39.94	2.38	2.44E-12
E2F7	0.35	1.96	2.49	2.48E-13
EMX1	0.14	0.85	2.61	2.48E-13
CBX2	0.36	2.70	2.89	2.66E-11
HOXA9	0.08	1.66	4.42	3.38E-10
HOXC11	0.09	2.37	4.79	6.18E-13
HOXC9	0.07	2.31	5.11	1.86E-14
SALL4	0.05	2.15	5.57	2.23E-14

FC, fold change; FDR, false discovery rate.

Screening of prognostic transcription factors

The differentially expressed transcription factors were used as potential prognostic factors for univariate Cox analysis by combining the expression of differentially expressed transcription factors with the overall survival status and overall survival time in the patient's clinical information. P<0.05 was used as the screening standard to screen the transcription factors related to the prognosis of patients with gastric cancer.

Construction of transcription factor interaction network

The interaction network between differentially expressed transcription factors was constructed with the String database. Key genes were determined according to the number of interactions between transcription factors.

Correlation between mutation and expression

Spearman test was used to explore the correlation between transcription factor mutation and gene expression.

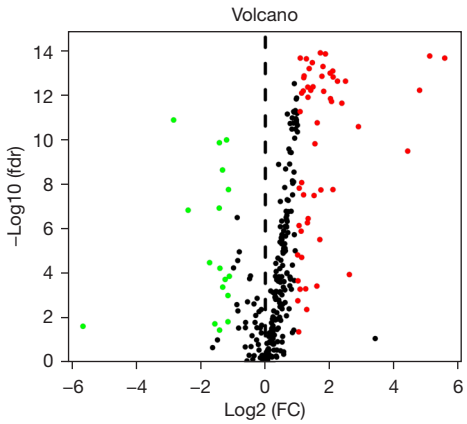

Figure 1 Transcription factors were differentially expressed in 32 normal tissues and 375 gastric cancer tissues in TCGA database. Red indicates up-regulation in gastric cancer, and green indicates down-regulation in gastric cancer. TCGA, the Cancer Genome Atlas; fdr, false discovery rate; FC, fold change.

Correlation between expression of transcription factors and chemosensitivity

GDSC database was used to explore the markers for predicting chemosensitivity. According to the expression of key genes in cancer cells, cancer cells were divided into high expression group and low expression group, and the half maximal inhibitory concentration (IC50) of high and low expression groups against different chemotherapeutic drugs was compared.

Statistical analysis

All data were statistically analyzed by R software (MathSoft, USA) and related R package in this study. P<0.05 indicates that it is statistically significant.

Results

Differential transcription factor

Taking | log2FC | ≥1 and FDR <0.05 as the screening conditions, the expression of transcription factors in 32 normal tissues and 375 gastric cancer tissues were compared. A total of 17 differentially expressed transcription factors were screened. Compared with normal tissues, 15 transcription factors were upregulated and 2 transcription

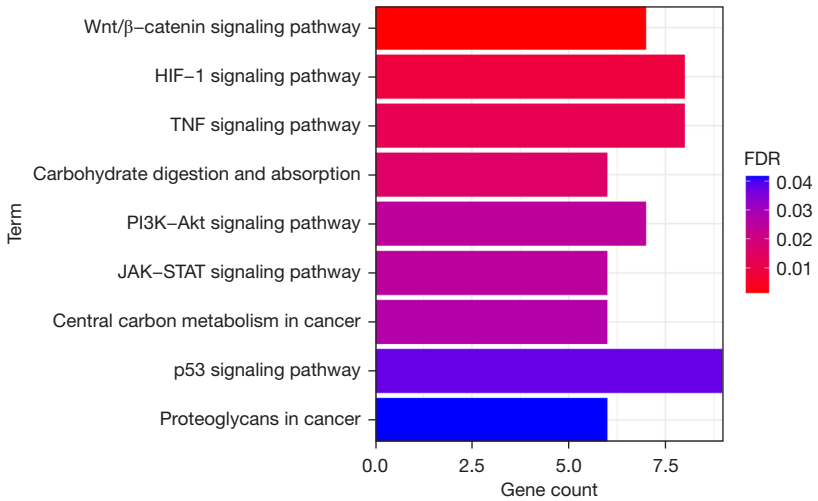

Figure 2 Enrichment analysis of differentially expressed transcription factor KEGG pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

Table 2 Differentially expressed transcription factors associated with prognosis

ID	HR	95% CI		P value
		Lower	Upper	
CENPA	1.42	1.04	2.18	0.002
E2F1	2.16	1.08	3.14	0.003
EMX1	1.89	0.93	2.33	0.014
HOXA9	2.79	1.65	3.47	0.026
FOXM1	2.46	1.4	3.15	0.037
MYBL2	1.01	0.83	1.26	0.041
RXRG	0.88	0.51	1.14	0.001
SOX4	0.67	0.49	0.94	0.021

HR, hazard ratio; CI, confidence interval.

factors were downregulated in gastric cancer tissues, as shown in *Table 1*. The volcano diagram of differentially expressed transcription factors is shown in *Figure 1*.

Enrichment analysis of differentially expressed transcription factor in the KEGG pathway

The differentially expressed transcription factors were enriched and analyzed with the KEGG database. Differential transcription factors were significantly enriched in *Wnt/β-catenin* signaling pathway, *HIF-1* signaling pathway, *TNF* signaling pathway, central carbon metabolism in cancer, *PI3K-Akt* signaling pathway, *JAK-STAT* signaling pathway, carbohydrate digestion and absorption, *p53* signaling pathway, and proteoglycans in cancer (*Figure 2*).

Prognosis related genes

This study combined the expression of differentially expressed transcription factors with the overall survival status and overall survival time in patients' clinical information. It used differentially expressed transcription factors as potential prognostic factors for univariate Cox analysis. Taking P<0.05 as the screening criterion, a total of 8 transcription factors were prognostic factors. Among them, *CENPA*, *E2F1*, *EMX1*, *HOXA9*, *FOXM1*, and *MYBL2* were prognostic risk factors (HR >1; P<0.05), and *RXRG* and *SOX4* were prognostic protective factors (HR <1; P<0.05; *Table 2*).

Construction of differentially expressed transcription factor interaction network

Differentially expressed transcription factors in normal tissues and gastric cancer tissues interacted, as shown in *Figure 3*. *FDXM1* interacted with *E2F7*, *MYBL2*, *E2F1*, *NCAPG*, and *SOX9*. Using the number of interacting transcription factors as the screening criteria, *FOXM1* was the most widely interacted gene in the differentially expressed transcription

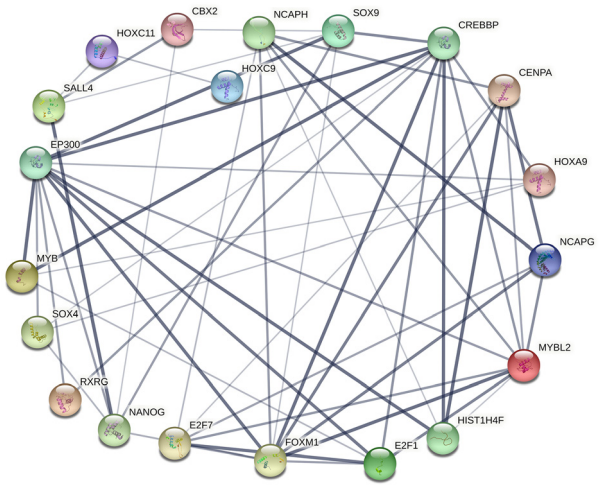

Figure 3 Construction of differentially expressed transcription factor interaction network.

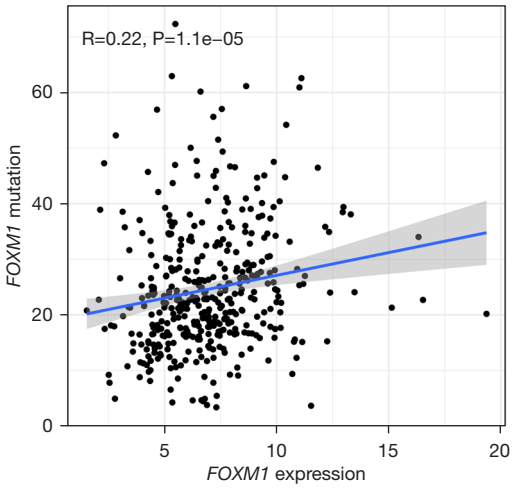

Figure 4 Correlation between *FOXM1* gene expression and gene mutation in gastric cancer.

factor network associated with the prognosis of gastric cancer.

Correlation between FOXM1 transcription factor mutation and expression

Since the above results indicated that *FOXM1* was the most widely interacting transcription factor, it was considered as a potential biomarker for gastric cancer, and the relationship between its mutation and expression was further investigated. *FOXM1* gene mutation was positively correlated with mRNA expression (R =0.22; P<0.05), as shown in *Figure 4*.

FOXM1 expression and chemosensitivity

Based on the median value of *FOXM1*, the gastric cancer cells were divided into *FOXM1* high-expression group and low-expression group. There was no significant difference in IC50 of 5-fluorouracil between the *FOXM1* high expression group and the low expression group (P>0.05). The IC50 of paclitaxel in the *FOXM1* high expression group was significantly higher than that in the *FOXM1* low expression group (P<0.001). The IC50 of cisplatin in the *FOXM1* high expression group was significantly higher than in the *FOXM1* expression group (P<0.05; *Figure 5*).

Discussion

The mechanism of drug resistance in gastric cancer is very complex. Mechanisms such as intracellular drug efflux and intracellular drug redistribution, changes in the level of intracellular drug-targeted enzymes, and enhanced cellular repair DNA damage can enhance the drug resistance of gastric cancer cells. Changes in the tumor microenvironment and the expression of therapeutic targets may play a key role in the drug resistance of gastric cancer. It is currently believed that some genes may be associated with drug resistance in gastric cancer, including MDR1, MRP1, mTOR and HIF-1α (8). Exploring the relationship between the expression of key pathogenic genes in gastric cancer and the sensitivity to chemotherapeutic drugs can avoid gastric cancer drug resistance and provide a basis for clinical chemotherapeutic drug selection.

Our results showed that the differentially expressed transcription factors were significantly enriched in the gene pathway of the *Wnt/β-catenin* signaling pathway, the *PI3K-Akt* signaling pathway, and the *p53* signaling pathway, which are a common cancer pathogenic signaling pathway. Existing research confirmed that miRNA, mRNA and lncRNA promote or inhibit tumor progression by activating or inhibiting these pathways (9-14). Oliveira *et al.* (9) compared the difference of protein expression on the Wnt pathway in 72 gastric cancer specimens with the immunohistochemical method. This study confirmed that Wnt was involved in the progression of gastric cancer. Singh *et al.* (10) indicated that the *PI3K-Akt* signaling pathway is frequently activated in gastric cancer. The imbalance of this pathway leads to the occurrence of gastric cancer. Therefore, targeted treatment is needed to obtain more

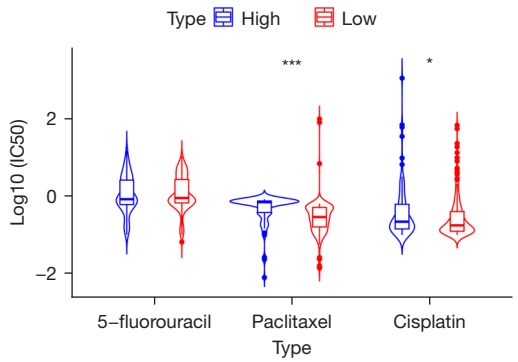

Figure 5 IC50 of chemotherapeutic drugs (5-fluorouracil, paclitaxel, and cisplatin) in *FOXM1* high expression group and low expression group were compared. ***P<0.001; *P<0.05.

effective anticancer therapy. Singh *et al.* (10) also used *PI3K-Akt* signaling pathway to predict biomarkers and promoted personalized cancer treatment and effectively inhibiting the *PI3K-Akt* signaling pathway. The *p53* signaling pathway is a common cancer pathogenic pathway (11,12), and its role in gastric cancer has also been widely confirmed (13,14). Our pathway enrichment analysis results demonstrated that the differentially expressed transcription factors we screened may play a key role in the occurrence and progression of gastric cancer.

FOXM1 was the key transcription factor of gastric cancer identified in this study. We found that *FOXM1* was highly expressed in gastric cancer, and its gene expression was positively correlated with gene mutation. We also found that *FOXM1* was a risk factor for the prognosis of patients with gastric cancer. These results suggest that *FOXM1* may promote the occurrence and progression of gastric cancer. A study pointed out that *FOXM1* is overexpressed in Pan-cancer, which is related to the occurrence and progression of most malignant tumors (15). The study showed that the expression of *FOXM1* was significantly increased at mRNA and protein levels in tumors with *FOXM1* mutation, *p53* inactivation and *Rb-E2F* abnormality (15). The results also illustrated that *E2F* and *cyclin E1* regulate *FOXM1* (15). It is important to point out that Barger *et al.* (15) found a mutation in *FOXM1* in a pan-cancer study, resulting in a significant increase in *FOXM1* expression, which is consistent with our conclusion. Furthermore, Wierstra (16) suggested that transcription factor *FOXM1* is overexpressed in many human cancers, including liver cancer, gastric cancer, prostate cancer, brain cancer, breast cancer, lung cancer, colon cancer, pancreatic cancer, cervical cancer, ovarian cancer, and nervous system cancer. *FOXM1* was involved in cell initiation, progression, metastasis, and the response to anti-cancer drugs.

We found that the IC50 of paclitaxel and cisplatin in the *FOXM1* high expression group was higher than that in the *FOXM1* low expression group. The results indicated that the higher the expression of *FOXM1*, the less sensitive the patients were to paclitaxel and cisplatin. There was no significant difference in the IC50 of *FOXM1* high expression group and low expression group to 5-fluorouracil. Our analysis suggested that *FOXM1* may not be involved in the induction of gastric cancer sensitivity to all chemotherapeutic drugs. Some existing studies also showed the relationship between *FOXM1* and chemoresistance, which is consistent with our results (17-20). For example, Okada *et al.* (17) identified 53 gastric cancer patients whose immunohistochemistry was *FOXM1* positive. Okada *et al.* (17) also found that *FOXM1* expression was an important independent prognostic risk factor for overall survival and disease-free survival in gastric cancer patients through multivariate analysis. The results of Okada *et al.* (17) also showed that *FOXM1* overexpression was significantly correlated with the drug resistance of docetaxel + 5-fluorouracil + cisplatin chemotherapy. In contrast, the 5-fluorouracil + cisplatin chemotherapy drug resistance was not significant in patients with advanced gastric cancer. Inhibiting overexpression of *FOXM1* was a promising strategy for the treatment of advanced gastric cancer. Li *et al.* (18) considered that *FOXM1* overexpression mediates the resistance of gastric cancer to docetaxel. They showed that *FOXM1* acts with the downstream target tubulin unstable protein stathmin to change microtubule dynamics to protect tumor cells from docetaxel-induced apoptosis. Their immunohistochemical analysis showed a correlation between the expression levels of *FOXM1* and stathmin in 103 postoperative specimens of gastric cancer. Li *et al.* (18) also found that, when *FOXM1* inhibitor sulfur chain protein was used to reduce the expression of *FOXM1*, the resistance of gastric cancer to docetaxel was reversed, and *FOXM1* and stathmin were downregulated. Therefore, *FOXM1* can be used as a useful marker for predicting and monitoring docetaxel response. Furthermore, docetaxel resistance can be reversed by inhibiting *FOXM1*. *FOXM1* may become a new therapeutic target for docetaxel-resistant gastric cancer. In other cancers, high expression of *FOXM1* also leads to increased resistance to chemotherapeutic drugs. Wang *et al.* (19) found that *FOXM1* expression was significantly correlated with cisplatin-based chemotherapy resistance

and poor prognosis in patients with advanced non-small cell lung cancer. Lin *et al.* (20) indicated that *FOXM1* participates in docetaxel resistance in castration-resistant prostate cancer by inducing *AMPK/mTOR* mediated autophagy. These findings support our findings. *FOXM1* plays an oncogenic role in most cancers and is associated with chemoresistance. However, our study is limited to data analysis from public repositories and requires validation of experimental data *in vivo* and *in vitro*.

In conclusion, we found that *FOXM1* is a transcription factor that is highly expressed in gastric cancer. The high expression of *FOXM1* is associated with paclitaxel and cisplatin resistance in patients with gastric cancer. Therefore, *FOXM1* is a potential target for the treatment of gastric cancer.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-274/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-274/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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