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Original Research Article

CYP4F3 is associated with poor prognosis and resistance to oxaliplatin-based chemotherapy in colorectal cancer

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Abstract

Purpose: *To screen the expression of different genes related to oxaliplatin resistance in colorectal cancer (CRC) therapy.*
Methods: *Limma and principal component analysis (PCA) techniques were used to determine genes with significantly different expression levels in the Gene Expression Omnibus (GEO) dataset. A lasso regression model and Venn diagram were used to analyze the intersecting genes. Gene Expression Profiling Interactive Analysis (GEPIA) and the University of Alabama at Birmingham Cancer data analysis Portal (UALCAN) online platform were used to analyze the expression of CYP4F3. The relationship between CYP4F3 expression and survival rate in colorectal cancer was analyzed by Kaplan–Meier curve, while the related pathways involving CYP4F3 were determined by Metascape and gene Ontology-Kyoto Encyclopedia of Genes and Genomes (GO-KEGG) analysis. Furthermore, the correlation between CYP4F3 and TME-related cells was analyzed by Pearson score. In addition, analysis of clinically tested and FDA-approved drugs significantly associated with CYP4F3 was carried out using CellMiner database.*
Results: *PCA and volcano plot analysis indicated there are four upregulated genes and 11 down-regulated genes in oxaliplatin-resistant CRC cells. The intersection gene was CYP4F3 in the lasso regression model and differentially expressed genes (DEG). Moreover, CYP4F3 was upregulated and associated with poor survival in CRC. Gene set enrichment analysis (GSEA), KEGG enrichment, and PPI analysis showed that CYP4F3 and GNG3 are the most significant genes in oxaliplatin-resistant CRC. Furthermore, CYP4F3 expression negatively correlated with the enrichment of T cells, while the expression of CYP4F3 was not associated with drug sensitivity in CRC cells.*
Conclusion: *The findings of this study suggest that CYP4F3 may be a target for the treatment of oxaliplatin-resistant CRC. However, the underlying mechanism of CYP4F3 in the regulation of sensitivity to oxaliplatin needs further investigation.*

Keywords: *Colorectal cancer, CYP4F3, Gene Expression Omnibus Chip, Poor prognosis, Oxaliplatin-resistant*

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INTRODUCTION

Colorectal cancer is a leading cause of cancer-related deaths in the world. It is the second and third most commonly diagnosed cancer in women and men, respectively, with more than 1.2 million patients diagnosed each year. Therefore, it is essential to reveal its mechanism and find new therapeutic and prognostic targets is necessary for the development of effective treatments for patients with colorectal cancer. The application of molecular biomarkers in diagnosis, prognosis, and treatment is crucial to reduce cancer mortality [1]. With the advent of microarrays and high-throughput technologies, genome-wide expression profiling greatly helps in understanding basic cancer biology and the identification of new biomarkers related to tumor behavior and patient prognosis [2]. Cytochrome P450 family 4 (CYP4) enzymes are called microsomal omega (ω)-hydroxylases, which metabolize fatty acids, eicosanoids, vitamin D, and carcinogens [3]. These enzymes not only play a role in endogenous functions but also participate in the metabolism of a variety of carcinogens and anti-cancer drugs. Therefore, cytochrome p450 is considered to play an important role in tumor biology [4]. Specifically, CYP4 enzymes usually act as microsomal omega (ω)-hydroxylases, metabolize fatty acids, eicosanoids, and vitamin D, and play important role in chemical defense [5]. *CYP4F3* is a member of the CYP4F subfamily. A previous study reported that *CYP4F3* has a potential role in lung cancer [6]. Benzene metabolites induce *CYP4F3* expression in human promyelocytic leukemia cell lines, indicating that *CYP4F3* may be related to leukemia [7]. However, there are few studies on *CYP4F3*, and its functional mechanism is still unclear. In this study, Gene Expression Omnibus (GEO) chip data was used to screen differentially expressed genes (DEGs) and perform gene Ontology-Kyoto Encyclopedia of Genes and Genomes (GO-KEGG) enrichment analysis to determine the expression of *CYP4F3* in colorectal cancer (CRC) oxaliplatin-sensitive cell lines and resistant cell lines. The relationship between *CYP4F3* expression and patient survival prognosis was analyzed, and the clinicopathological correlation and prognostic significance of *CYP4F3* expression in oxaliplatin-resistant colorectal cancer patients were discussed.

METHODS

Data collection and analysis

Principal component analysis (PCA) was used as described and previously depicted. GSE77932 (DEGs from oxaliplatin-resistant cell lines—DLD1 and HCT116) was analyzed using R package limma analysis (cluster Profiler, org. Hs. eg. db package). Based on the volcano diagram, five differentially upregulated genes and 11 downregulated genes were identified by the intersection of differentially regulated genes. Then, four upregulated and four downregulated DEGs were selected for enrichment analysis and visualized them using cluster dendrogram and heatmap.

Feature screening of differentially expressed genes based on machine learning

Using the R language (R version 4.0.4, clusterProfiler package) feature selection Support Vector Machine Recursive Feature Elimination (SVM-RFE) algorithm, a lasso regression model was built to characterize the difference genes, obtain the intersection of the feature genes with the above heat map genes, and plot the intersection of DEG and lasso wenn diagramData filtering and standardization were performed as described previously. The heat map shows the top eight genes with the greatest differences. Regression analysis was used for all DEGs. Four significant genes were identified and intermixed with the above eight genes to determine the intersection genes [8].

Kaplan–Meier curve and UALCAN analyses

For survival analysis, the prognosis of patients was measured with online software using the Kaplan–Meier curve tool (http://kmplot.com/ analysis/). Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) and the University of Alabama at Birmingham Cancer data analysis Portal (UALCAN; http://ualcan.path.uab.edu/ analysis.html) online software was used to analyze the expression of *CYP4F3* in CRC.

Construction of drug-resistant cells and cell proliferation assay

Cells were exposed to different concentrations of oxaliplatin (L-OHP; 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 μg/mL) and treated for 6 months in continuous culture. Cell viability was performed as previously described [9]. A total of 1.0×10^3 cells (HCT116/L-OHP and LoVo/L-OHP cells were divided into four groups: control, *CYP4F3*, shNC, and shCYP4F3) and seeded into 96-well plates, after 24 h, the cells were treated with oxaliplatin for 48 h, then the cell proliferation rate was measured use MTT assay kit.

Colony formation assay

Cell colony formation was performed as previously described [10]. HCT116/L-OHP and LoVo/L-OHP cells (5 × 10^2 cells) were divided into four groups (control, *CYP4F3*, shNC, and shCYP4F3) and seeded into 60 mm dishes. The cells were cultured with regular medium including 5 μg/mL oxaliplatin for 48 h. Then the foci formation was measured using crystal violet staining, photographed, and counted.

Western blotting assay

Protein concentrations of whole cell lysates were determined using NanoDrop OneC, using Bio-Rad's protein detection reagents as described previously [10]. Equal amounts of whole-cell lysates were loaded by SDS-PAGE and immunoblotted with the indicated antibodies. The antibodies were used as follows: CYP4F3 (1:1000, GeneTex, GTX81119, USA) and GAPDH (1:1000, Santa, sc-47724, USA). Cells were collected, washed, and lysed using EBC buffer with protein inhibitor, and spun. Finally, blotting was performed on the membrane with ECL reagent and photographed.

Statistical analysis

Student's *t*-test was used in statistical analyses. Data are presented as mean ± standard error of the mean (SEM) of three independent experiments. A *p*-value of 0.05 or less was considered significant.

RESULTS

CYP4F3 is specific in oxaliplatin-resistant CRC cells

To compare DEGs in oxaliplatin-resistant CRC cells and oxaliplatin-sensitive CRC cells, the results of PCA analysis of DEGs were obtained after normalization of GSE77932, and the gene expression levels were found to be different in the two groups (Figure 1 A and B). Next, volcano plots were used to analyze the up- and down-regulation of genes in these two groups. Four up-regulated DEGs and 12 down-regulated DEGs were identified (Figure 1 C). Then four up-regulated genes and four down-regulated genes were selected for cluster dendrogram and heat map analysis (Figure 1 D and E). *CYP4F3* was one of the most significant DEGs in oxaliplatin-resistant CRC cells. Additionally, the study detected four feature genes using lasso regression model analysis (Figure 2 A and B). *CYP4F3* was the only intersection gene between the feature genes and heatmap-related DEGs (Figure 2 C). Collectively, these data suggest that *CYP4F3* is a feature gene in oxaliplatin-resistant DEGs.

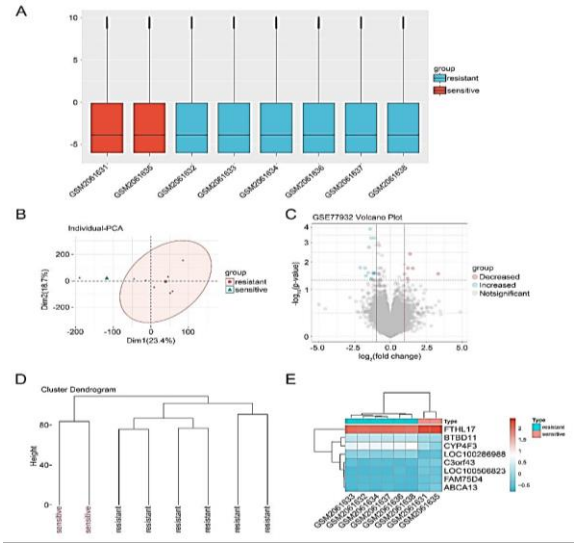

Figure 1: Analysis of differentially-expressed genes (DEGs) for oxaliplatin resistance in colorectal cancer cells based on GEO Chip. (A) PCA analysis was performed by downloading GSE77932 from the GEO database and removing batch effects. (B) PCA downscaling was first performed to analyze significant differences between resistant and sensitive samples in GEO data and to assess the independence of each group. (C) The limma package was first used to identify differentially expressed genes, and volcano plot analysis was performed to compare expression differences of screened genes ($|Log_2 FC| > 1$, $p < 0.05$). (D & E) Cluster analysis was performed on the differential genes obtained from the above analysis and a dendrogram of the clustering system was drawn, including two groups of sensitive and six groups of resistance (D). Heat maps were drawn to analyze the four data points with the most significant differences according to the comparison of differential genes (E)

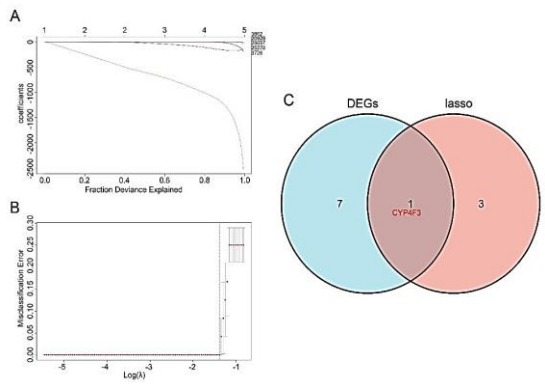

Figure 2: Feature screening of differentially-expressed genes based on machine learning. (A) characteristics of DEGs analyzed using the Lasso regression model. (B) Venn diagram analysis of the intersection genes between DEGs and the Lasso model

Increased expression of *CYP4F3* in colorectal cancer was associated with poor prognosis

Next, the study analyzed the expression of *CYP4F3* in CRC. As shown in Figure 3 A, *CYP4F3* was highly expressed in tumor samples compared to normal tissues by UALCAN analysis. Additionally, the expression of *CYP4F3* was upregulated in adenocarcinoma and mucinous adenocarcinoma. For the nodal metastasis status, *CYP4F3* was highly expressed in colon adenocarcinoma (N0, N1, and N2) when compared to the normal group (Figure 3 A). Moreover, *CYP4F3* expression was upregulated in tumors when compared with normal cells by GEPIA analysis (Figure 3 B). Then, using the GEPIA online platform to analyze The Cancer Genome Atlas data, as shown in Figure 3 C, there was no significant difference between high *CYP4F3* and low *CYP4F3* expression in overall survival. However, high expression of *CYP4F3* in rectum adenocarcinoma was associated with poor survival using Kaplan–Meier Plotter analysis (Figure 3 D). Taken together, these results suggest that *CYP4F3* overexpression may be an indicator of poor survival in CRC.

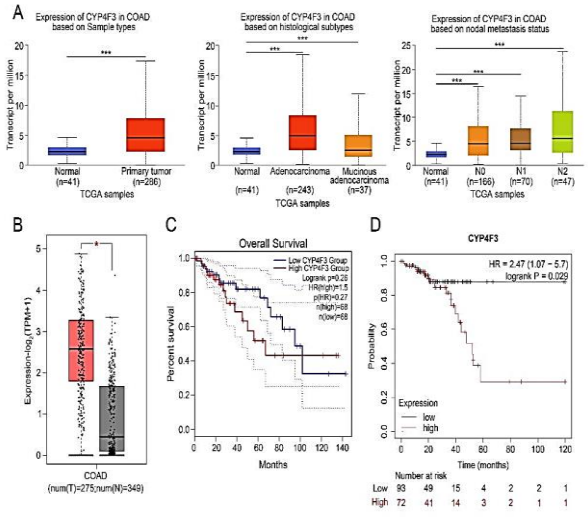

Figure 3: Increased expression of *CYP4F3* in colorectal cancer is associated with poor prognosis. (A & B) The expression of *CYP4F3* was analyzed using GEPIA and UALCAN online platforms. (C & D) Kaplan–Meier curve of the association between *CYP4F3* mRNA expression and the prognosis of CRC patients

***CYP4F3* promoted colorectal cancer cell growth and oxaliplatin resistance**

Next, the study analyzed the expression of *CYP4F3* in CRC cells. As shown in Figure 4 A, *CYP4F3* was more highly expressed in HCT116, LoVo, HCT116/L-OHP, and LoVo/L-OHP cells than in NCM460 cells. Knockdown of *CYP4F3* inhibited viability of HCT116 and LoVo cells, while overexpression of *CYP4F3* increased cell viability in the cell lines (Figure 4 B and C). Moreover, in these cell lines, *CYP4F3* deficiency reduced foci formation, and cells with *CYP4F3* overexpression showed increased foci formation (Figure 4 D).

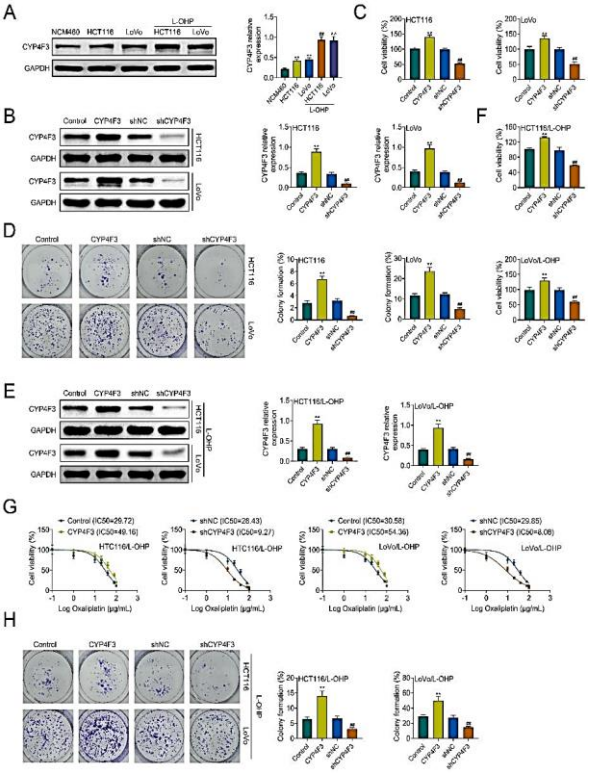

Figure 4: *CYP4F3* promotes colorectal cancer cell growth and oxaliplatin resistance. (A) The expression of *CYP4F3* in CRC cells (HCT116, LoVo, HCT116/L-OHP, LoVo/L-OHP) was measured using western blotting. (B) HCT116 and LoVo cells were treated with *CYP4F3*, and shCYP4F3, and the expression of *CYP4F3* in the cell lines was measured by western blotting. (C & D) HCT116 and LoVo cells were seeded into 96-well plate and a 6-well plate, respectively. After 48 h, cell viability was conducted by MTT assay. (C) For colony formation assay, cells were cultured for 2 weeks, then the colony was counted (D). (E) HCT116/L-OHP and LoVo/L-OHP cells were transfected with the indicated plasmids. The expression of *CYP4F3* in the cell lines was measured as in (A). (F & G) Cell viability and cell proliferation of the cell lines were conducted by MTT assay and colony formation assay as in C & D. Data are representative of three independent experiments (mean ± SD). $^{**}p < 0.01$; $^{\#\#}p < 0.01$, *represents CYP4F3 overexpression group compared to the control group; $^{\#}$represents CYP4F3 knockdown group compared with the shNC group

Similarly, cell proliferation and lesion formation were analyzed after CYP4F3 or shCYP4F3 treatment in HCT116/L-OHP and LoVo/L-OHP

cell lines. (Figure 4 E). As shown in Figure 4F, *CYP4F3* deficiency increased the sensitivity of HCT116/L-OHP and LoVo/L-OHP cells to oxaliplatin, while overexpression of *CYP4F3* reduced the sensitivity of HCT116/L-OHP and LoVo/L-OHP cells to oxaliplatin. In addition, *CYP4F3* induced colony formation in HCT116/L-OHP and LoVo/L-OHP cells with oxaliplatin treatment (Figure 4 G). Collectively, these data suggest that *CYP4F3* induced colorectal cancer cell growth and oxaliplatin resistance.

DISCUSSION

Colorectal cancer is the third most common malignancy and the second highest cause of mortality amongst all types of cancer. It accounts for 10.2 % of morbidity and 9.2 % of mortality globally, amongst all kinds of cancer types. The morbidity and mortality of CRC have increased in the past two decades. Therefore, it is essential to clarify the molecular mechanisms involved in CRC progression. In this study, *CYP4F3* downregulation at the intersection of DEGs and the lasso model in oxaliplatin-resistant CRC, relative to oxaliplatin-sensitive CRC. Moreover, *CYP4F3* was highly expressed and associated with poor survival in rectum adenocarcinoma. The DEGs between the oxaliplatin-resistant and -sensitive group were mainly involved in cell-substrate junction and transporter complex. These DEGs were also negatively correlated with TME, especially the enrichment of T cells. In addition, the expression of *CYP4F3* was significantly negatively correlated with the z-score of Ixazomib citrate, bortezomib, midostaurin, pazopanib, vismodegib, and arsenic trioxide, and exerted no effect on the sensitivity of these drugs.

CYP4F3, a member of the cytochrome P450 family, played an important role in catalyzing the oxidation of fatty acid epoxides [11]. The deficiency of CYP4F3 leads to growth arrest and cell death in HL60 cells [12]. In this study, CYP4F3 also had a positive regulatory effect on colony formation in CRC cells. In addition, CYP4F3 is associated with celiac disease, with symptoms manifesting as an inflammatory state and an impaired intestinal barrier [13]. CYP4F3 is associated with the risk of Crohn's disease, leaving the intestine in an inflammatory state and leading to an increased risk of colon cancer in patients [14]. Valérie *et al* [15] demonstrated that CYP4F3 is involved in metabolizing white matter toxins and leukotriene B4, suggesting a possible role in regulating inflammatory cellular responses play an important role in regulation of inflammatory cellular responses. The catalytic activity of CYP4F3B (the component of *CYP4F3*) was similar to that observed in human liver microsomes. Exposure of differentiated HepaRG cells to various fatty acids induces an adaptive response to hepatocyte steatosis and is also involved in reducing the production of new fat [16]. A previous study demonstrated that PGA1 upregulated the expression of CYP4F3B in HepaRG cells [17]. Gandhi and colleagues reported that *CYP4F3* was upregulated in pancreatic ductal adenocarcinoma [18]. In this study, *CYP4F3* expression was upregulated in CRC. The reasons for increased *CYP4F3* expression remain to be identified.

CONCLUSION

CYP4F3 is a marker gene in oxaliplatin-resistant CRC, with upregulated expression and correlation with poor survival in CRC. It positively correlates with the proliferation and foci formation of CRC cells. Furthermore, *CYP4F3* plays an oncogenic role in the regulation of cell proliferation and foci formation in HCT116 and LoVo cells. The expression of *CYP4F3* positively associates with the sensitivity of HCT116/L-OHP and LoVo/L-OHP to oxaliplatin. However, the underlying mechanism of *CYP4F3* in the regulation of sensitivity to oxaliplatin needs further investigation.

DECLARATIONS

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None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this

article will be borne by the authors. Shifen Zhang and Yuxiang Fu designed the study and supervised the data collection. Liming Liu analyzed and interpreted the data. YaJie Yang and Juan Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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