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Original Research Article

IFI6 predicts prognosis and promotes cell growth of human colorectal cancer

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Abstract

Purpose: *To analyze the effect of IFI6 in colorectal cancer (CRC).*
Methods: *Differentially-expressed genes were analyzed using a volcano plot. Metascape and STRING were used for functional enrichment and protein-protein interaction network analyses, respectively. Expression of IFI6 was analyzed using the online platforms TIMER, UALCAN, and GEPIA. The correlation between IFI6 and poor prognosis was analyzed using prognoscan, while Western blotting was used to determine the expressions of related proteins. Cell proliferation was performed by CCK8 and foci formation assays. Cell apoptosis was assessed using flow cytometry.*
Results: *Differentially-expressed genes between CRC cells and alisertib-resistant CRC cells comprised 84 upregulated genes and 358 downregulated genes ($p < 0.05$). Enrichment analysis showed that differential genes were mainly involved in interferon α/β signaling, antiviral mechanism of interferon-stimulated genes, and positive regulation of the immune response. IFI6, a hubgene in the protein-protein interaction network, was highly expressed in CRC ($p < 0.001$) and correlated with poor prognoses ($p = 0.028$). IFI6 knockdown inhibited cell viability and colony formation and induced cell apoptosis by upregulation of p53, p21, and Bax.*
Conclusion: *These results suggest that IFI6 may be a biomarker for the diagnosis of human CRC.*

Keywords: *IFI6, Colorectal cancer, Cell viability, Immune infiltration, Poor prognosis*

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies of the digestive tract, causing more than 6 million deaths each year. Despite the rapid development of various therapeutic methods including surgery, radiotherapy, chemotherapy, and immunotherapy, the poor prognosis of CRC patients is still a difficult problem to overcome. Increasingly, studies have shown that abnormal regulation of genes, including activation of oncogenes and inhibition of tumor suppressor genes, and activation or inhibition of tumor-related signaling pathways affect the occurrence and development of CRC. There is a great need for a more comprehensive and systematic understanding of the molecular mechanism underlying the development of CRC, and the

identification of more specific clinical screening markers [1].

Interferon alpha (IFNα) - inducible protein 6 (IFI6) was first discovered using *in silico* analysis, along with three other genes, IFI27, ISG12b and ISG12c, which together constitute the human FAM14 gene family. However, the functions of these genes have not been fully resolved, and little is known about their physiological roles. Functionally, these genes mainly participate in immune regulation and anti-apoptotic functions by regulating the JAK/STAT signaling pathway, thereby blocking mitochondrial release of cytochrome c and terminating apoptosis. IFI6, a pro-survival protein, antagonizes trail-induced apoptosis in human myeloma cells [2,3]. It has been confirmed that IFI6 is increasingly expressed in esophageal squamous cell carcinomas. Knockdown of IFI6 inhibits cancer progression [4], promote breast cancer metastasis, and affect breast cancer prognosis and drug resistance [5,6].

However, the function of IFI16 in human colorectal cancer was poorly understand. This research mainly focuses on clarifying the critical role of IFI16 in CRC.

METHODS

Gene expression analysis

The expression of IFI6 in CRC was analyzed using a GEO dataset (GSE160037). The expression of IFI6 in CRC was analyzed using online platforms, including TIMER (https://cistrome.shinyapps.io/timer/), UALCAN (http://ualcan.path.uab.edu/analysis.html), and GEPIA (http://gepia.cancer-pku.cn/).

Gene ontology (GO)-KEGG enrichment analysis and protein-protein interaction (PPI) analysis

The TCGA-COAD dataset was used to draw survival prognosis curve. The TCGA_GTEx-COAD and TCGA-COAD datasets were used to conduct receiver operating characteristic (ROC) curve analysis.

Kaplan-Meier and ROC analysis

The TCGA-COAD dataset was used to draw the survival prognosis curve.

Correlation analysis

Correlation analysis was performed as previously described [7,8]. The correlations between KIF21B and genes associated with immune infiltration were analyzed with Pearson's score. Data was from TIMER.

Western blotting

Cells were seeded into 60 mm dishes, then washed using PBS, lysated by RIPA buffer with proteinase inhibitor to extract proteins. Protein samples was performed as previously described for gel running, transferring and incubating [9]. The antibodies used were as follows: IFI16 (sc-8023; 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), p53 (sc-8432; 1:1,000, Santa Cruz Biotechnology), BAX (sc-20067; 1:1,000, Santa Cruz Biotechnology), p21 (sc-6246; 1:1,000, Santa Cruz Biotechnology), and GAPDH (sc-47724; 1:1,000, Santa Cruz Biotechnology).

Cell viability and apoptosis analysis

The survival analysis of CRC cell lines was performed as previously described [9]. The CCK8 assay was used to analyze cell viability. For cell apoptosis, cells were collected, then washed using PBS, and incubated with PI and annexin V, final analyzed using flow cytometry.

Colony formation assay

For the clonogenic assay, 1×10^3 cells were seeded in 35-mm cell culture dishes. After 96 h, the colonies were fixed with 70 % ethanol for 30 min, stained, and counted using a microscope.

Statistical analysis

Student's *t*-test was used for statistical analysis. Data are presented as mean ± standard error of the mean (SEM) of three independent experiments. A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Differential gene expression in CRC cells

In order to screen for differential gene expression in CRC in the GEO dataset, the differentially-expressed genes (DEGs) between parental and alisertib resistance cells of CRC were analyzed using batch corrections (Figure 1 A). The expression of these genes was analyzed using volcano plot. Figure 1 B and C show that there were 84 upregulated DEGs and 358 downregulated DEGs between parental and alisertib resistance cells.

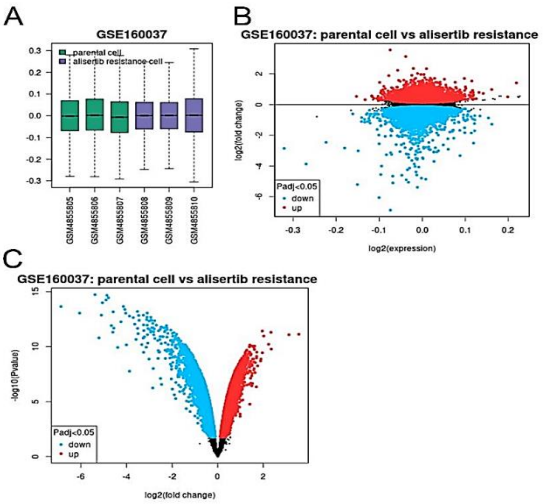

Figure 1: Analysis of differential gene expression in colorectal cancer cells (parental cells and alisertib resistance cells) based on GEO data. (A) Parental cells were divided into three groups (GSM4855805, GSM4855806, and GSM4855807), and alisertib resistance cells were also divided into three groups (GSM4855808, GSM4855809, and GSM4855810). A box plot was used to analyze gene expression in GSE160037. (B and C) A volcano map was used to visualize the differential genes between colorectal cancer cells and alisertib-resistant colorectal cancer cells

Differential gene enrichment

Differential genes with fold-change ≥ 2 were selected for enrichment analysis. Figure 2 A – C show that DEGs were involved mainly in IFNα/β signaling pathway, antiviral signaling pathway of IFN-stimulated genes, positive regulation of immune responses, NOTCH1 regulation of endothelial cell calcification, senescence and autophagy in cancer, regulation of peptidase activity, the p53 transcriptional gene network, and the p53 downstream pathway. Additionally, there were eight DEGs, (IFI44L, OAS1, IFITM1, BST2, ISG15, IFIT1, DDX58, and MX1) which interacted with IFI6, and IFI6 was a hubgene in the PPI network (Figure 2 D).

IFI6 was overexpressed in CRC and was associated with poor prognosis

Since IFI16 was a hub gene in DEGs, the expression of IFI6 in CRC tissues was analyzed by bioinformatics method. Figure 3 A shows that IFI6 expression was upregulated in tumor tissues from the TCGA-COAD dataset compared with matched normal tissues from TIMER analysis. Moreover, based on the sample types, IFI6 was also overexpressed in primary tumors (n = 286) when compared with normal specimens (n = 41) (Figure 3 B). In addition, IFI6 expression was increased in tumor specimens (n = 275) relative to normal specimens (n = 349) from the TCGA-COAD dataset (Figure 3 C). Collectively, these data suggest that IFI6 is highly expressed in CRC.

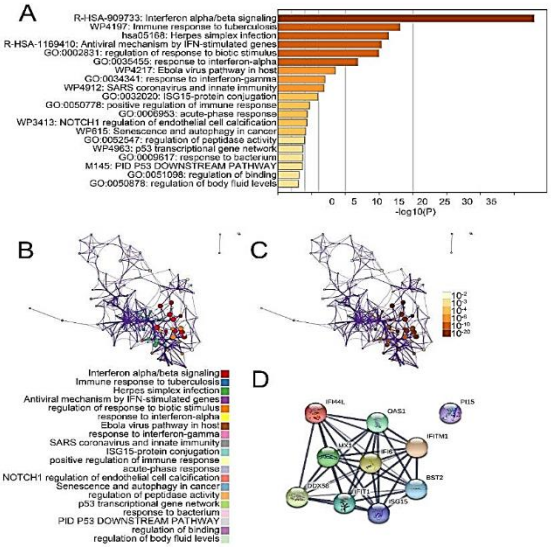

Figure 2: Differential gene enrichment analysis. (A) Metascape was used to analyze functional enrichment. A bar graph of enriched terms across input gene lists, colored with P-values. (B and C) Network of enriched terms: (B) colored via cluster identification, where nodes that share the same cluster identification are typically close to each other; (C) colored by p-values, where terms containing more genes tend to have more significant p-values. (D) STRING analysis of the protein-protein interaction network

Furthermore, Prognoscan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) was used to analyze the relationship between IFI6 expression and patient survival in the TCGA-COAD dataset. Figure 3 D shows that high expression of IFI6 (n = 30) was positively associated with poor survival in CRC (p = 0.028).

Correlation between IFI6 and immune infiltration of CRC cells

To clarify the function of IFI6 in CRC, the relationship between IFI6 and immune infiltration was analyzed using the TIMER data. As shown in Figure 4, the expression of KIF21B was positively associated with the infiltration level of cells, including B cells (cor = 0.119, p = 1.7e-2), CD4+ T cells (cor = 0.169, p = 6.72e-4), CD8+ T cells (cor = 0.158, p = 1. 44 e-3) and macrophage number (cor = 0.213, p = 1.64e-5), neutrophil granulocytes (cor = 0.395, p = 1.87e-16) and dendritic cells (cor = 0.326, p = 2.03e-11). In addition, the expression of KIF21B was negatively associated with the infiltration level of purity cells (cor = -0.265, p = 6.04e-8).

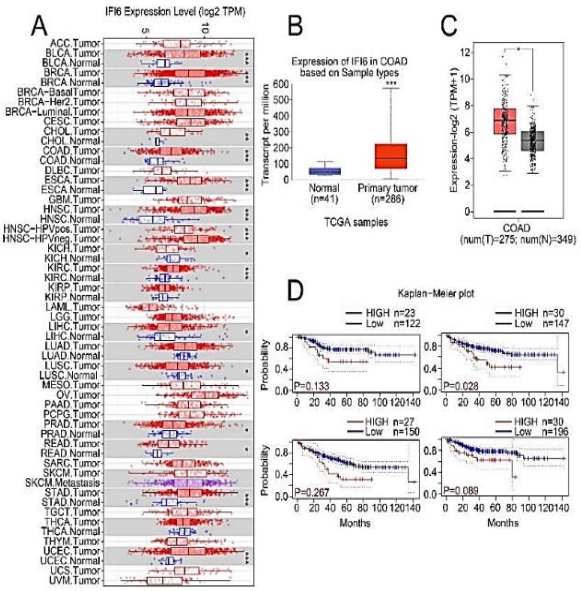

Figure 3: IFI6 was highly expressed in colorectal cancer and is associated with poor prognosis. (A-C) The expression of IFI6 in colorectal cancer was analyzed using TIMER, UALCAN, and GEPIA online platforms. (D) The correlation between IFI6 expression and patient survival was measured using a Kaplan-Meier plot

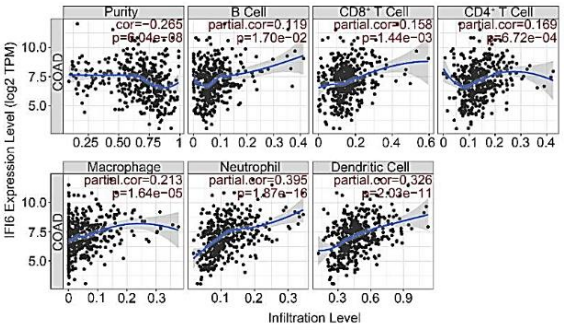

Figure 4: Relationship between IFI6 and immune infiltration of colorectal cancer cells. The correlations between IFI6 and immune infiltration (including purity, B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells) of colorectal cancer cells were analyzed via Pearson's score

IFI6 deficiency inhibited cell growth in CRC cells

Analysis of bioinformatics-related data showed that IFI6 was highly expressed in CRC tissues and correlated with poor survival. First, the expressions of IFI6 in normal colonic mucosa (NCM460) and CRC cells (SW480, SW620, HCT116, and SW837) was measured. Figure 5 A shows that IFI6 expression was upregulated in CRC cells when compared with normal colonic mucosa. Figure 5 B shows that knockdown of IFI6 reduced cell proliferation of HCT116 and SW480 cells when compared with control cells. Moreover, colony formation was inhibited after IFI6 knockdown in HCT116 and SW480 cells (Figure 5 C).

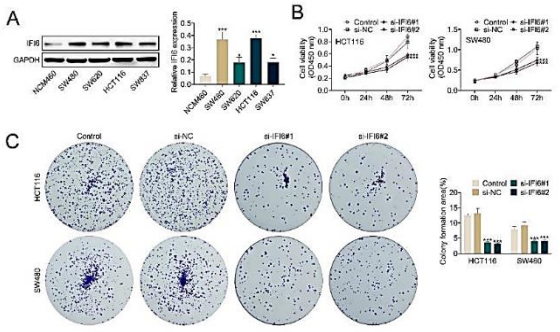

Figure 5: IFI6 enhanced the growth of colorectal cancer. (A) The expressions of IFI6 in normal colonic mucosa (NCM460) and colorectal cancer cells (SW480, SW620, HCT116, and SW837) were measured using western blotting. (B) SW480 and HCT116 cells were divided into four groups: control, si-NC, si-IFI6#1, and si-IFI6#2. Proliferation of these cells was measured using a CCK8 assay. (C) Colony formation assays were used to measure cell growth of SW480 and HCT116 cells after si-RNA treatment

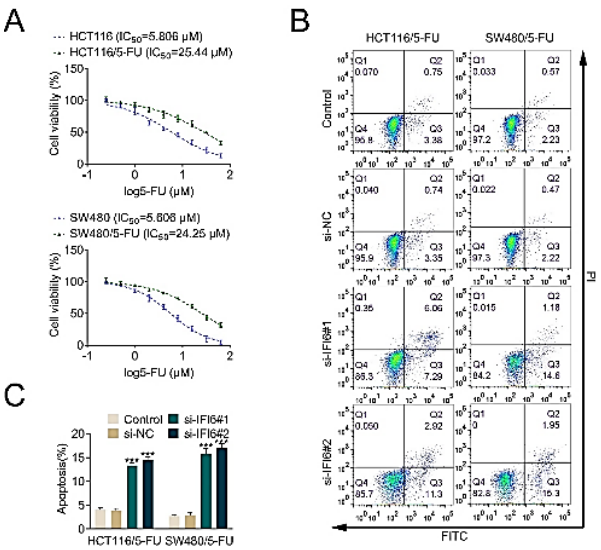

Figure 6: Knockdown of IFI6 increased apoptosis in 5-FU resistant cells. (A) The IC_{50} of 5-FU in 5-FU resistant HCT116 and SW480 cells was measured using the CCK8 assay. (B and C) Flow cytometry was used to measure cell apoptosis in 5-FU resistant HCT116 and SW480 cells. Data are representative of three independent experiments (mean ± SD). ***P < 0.001

IFI6 deficiency increased cell apoptosis in 5-FU resistant CRC cells

Figure 6 A shows that 5-FU resistant cells had a higher IC_{50} than parental HCT116 (25.44 μt vs. 5.806 μ5) and SW480 (24.25 μ5 vs. 5.606 μ5) cells and knockdown of IFI16 in 5-FU resistant

HCT116 and SW480 cell lines. Figures 6 B and C show that IFI6 deficiency significantly increased apoptosis in 5-FU resistant HCT116 and SW480 cells.

IFI6 deficiency inhibited cell growth in CRC cells

The expressions of p53, p21, and Bax were increased in HCT116 and SW480 cells after si-IFI6 treatment (Figure 7). These data indicate that IFI6 may negatively regulate cell apoptosis through the p53/p21 axis.

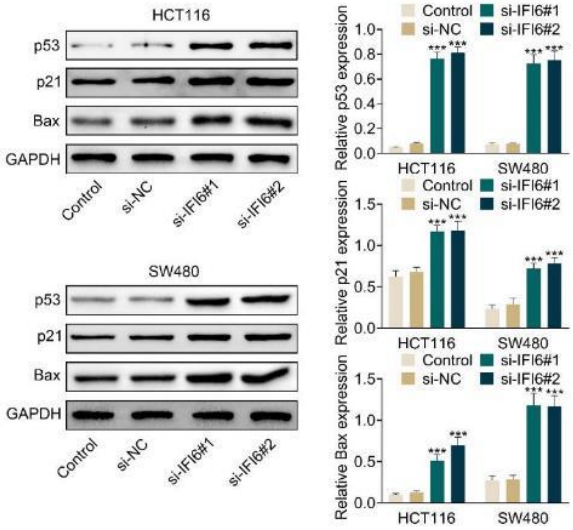

Figure 7: IFI6 deficiency induced the p53 signaling pathway. Expression levels of p53, p21, and Bax in HCT116 and SW480 cells treated with siRNA were measured using western blotting. Data are representative of three independent experiments (mean ± SD). ***$P < 0.001$

DISCUSSION

Colorectal cancer is the third most common malignancies and the second most common mortality in different types of cancers. Worldwide, it accounts for 10.2 % morbidity and 9.0 % mortality in all types of cancer [10]. With increasing world population, the morbidity and mortality of CRC have increased yearly during the past three decades. It is therefore essential to identify key biomarkers of poor survival involved in the regulation and progression of CRC. In the present study, there were 84 upregulated genes and 358 downregulated genes between parental cells and alisertib resistance CRC cells. These genes were mainly involved in IFN α/β signaling, antiviral mechanisms by IFN-stimulated genes, positive regulation of immune responses, NOTCH1 regulation of endothelial cell calcification, senescence and autophagy in cancer, regulation of peptidase activity, the p53 transcriptional gene network, and the p53 downstream pathway. IFI6 is a hub gene, which showed increased expression in CRC and correlated with poor prognosis. IFI6 depletion inhibited cell viability and colony formation and induced cell apoptosis by upregulation of p53, p21, and Bax.

Recently, studies have shown that IFI6 plays important roles in different types of cancers, including lung adenocarcinoma [11], myeloma [2], gastric cancer [12], oral squamous cell carcinoma (OSCC) [13], pancreatic cancer [14], breast cancer [5], prostate cancer [15], and ovarian cancer [16]. A previous study reported that IFI6 is a downstream gene of AGR2 playing critical role in lung carcinoma migration and that IFI6 is a key gene in the progression and development of OSCC [11]. The IFI6 is highly expressed in esophageal squamous cell cancer and is associated with poor prognosis [4]. The present study also showed that IFI6 expression was upregulated in CRC.

Functionally, a previous study reported that IFI6 deficiency reduces cell growth and facilitated cell apoptosis by increasing levels of reactive oxygen species (ROS) [4]. In gastric cancer, IFI6 negatively regulates cell apoptosis by decreasing regulation of caspase-3 [12]. In human myeloma cells, IFI6 reversed the anti-apoptosis effects of TRAIL [2]. LncRNA CTD-3252C9.4 promoted cell apoptosis and decreases cell proliferation by decreasing the transcription of IFI6 in pancreatic cancer. In ovarian cancer, IFI6 depletion constrained cell growth and increases cisplatin-induced apoptosis through the NF-κB pathway [14]. Similarly, the present study identified the same function of IFI6 in the regulation of cell growth and apoptosis in CRC. Here, IFI6 depletion induced cell apoptosis in CRC by upregulation of p53/p21 signaling. However, the underlying molecular mechanism needs further study. In addition, it has been shown that IFI6 is a target of ATF3 which exerts a tumor suppressor role in tongue squamous cell carcinomas by downregulation of IFI6 [17]. IFI6, (an IFN-inducible gene) shows reduced replication of the p7 mutant virus, with no effect on replication of the p7 wide-type virus. Moreover, IFI6 deficiency antagonizes the hypersensitivity of IFN to p7 mutant virus [18].

CONCLUSION

IFI6 associates with immune infiltration of CRC cells. However, IFI6 is a hub gene, which is associated with poor survival. Hence, IFI6 positively regulates cell viability and colony formation, and negatively regulates cell

apoptosis through downregulation of p53/p21 axis. Thus, IFI6 may be a marker gene for the diagnosis of human CRC.

DECLARATIONS

Acknowledgements

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Funding

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. Chu Lv and Meng Li designed the experiments, conducted them, analyzed and interpreted the data, and prepared the manuscript.

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