C6orf15 acts as a potential novel marker of adverse pathological features and prognosis for colon cancer

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Abstract

Objective To investigate the expression of chromosome 6 open reading frame 15 (C6orf15) in colon cancer and its effects on clinicopathological features and prognosis.

Methods Using the transcriptome and clinical data of colon cancer and normal tissues in The Cancer Genome Atlas (TCGA) database, the expression of C6orf15 mRNA in colon cancer samples and its relationship with clinicopathological characteristics and prognosis were explored. The expression level of C6orf15 protein in 23 colon cancer tissues was detected by immunohistochemistry (IHC). The possible mechanism of C6orf15 involved in the occurrence and development of colon cancer was explored by gene set enrichment analysis (GSEA).

Results Compared with normal tissues, C6orf15 was highly expressed in colon cancer (1.207±0.694 vs. 0.276±0.166, t=8.281, P<0.01). The expression level of C6orf15 was associated with tumor invasion depth (χ²=8.30, P=0.04), lymph node metastasis (χ²=36.97, P<0.001), distant metastasis (χ²=8.69, P=0.003), pathological stage (χ²=34.17, P<0.001). High expression of C6orf15 was associated with poor prognosis (χ²=6.43, P<0.05). The results of GSEA showed that C6orf15 promotes the occurrence and development of colon cancer by promoting the ECM receptor interaction pathway, Hedgehog signaling pathway and Wnt signaling pathway. Immunohistochemical results showed that the expression of C6orf15 protein in colon cancer tissues was correlated with the depth of invasion (P=0.023) and lymph node metastasis (P=0.048).

Conclusion C6orf15 is highly expressed in colon cancer tissue and is related to adverse pathological features and poor prognosis of colon cancer. It is involved in multiple oncogenic signaling pathways and may serve as a prognostic marker of colon cancer.

Introduction

Colorectal cancer (CRC) is one of the most common cancers both in men and women, CRC has the third highest estimated number of deaths of all cancer types in 2021[1]. Meanwhile, the incidence rates of CRC among the groups aged 20 to 39 years increased these years, which may be caused by obesity and changes in dietary factors[2]. In China, the cancer burden is still high and CRC was the second most common cancer among urban residents[3]. Exploring new markers and therapeutic targets has always been of considerable interest.

Chromosome 6 open reading frame 15 (C6orf15), also known as simian taste bud-specific gene (STG), is located at 6p21 in human being[4]. The exact function of STG has not been elucidated. In this study, for the first time, we report the C6orf15 expression level and its relationship with clinicopathologic features of colon cancer and to explore the role of C6orf15.

Results
C6orf15 mRNA was highly expressed in colon cancer

After removing duplicate samples, a total of 456 colon cancer tissues and 41 normal tissues were included in the analysis, including 41 paired tumor/non-tumor samples. The average expression levels of C6orf15 in colon cancer tissues were significantly higher than normal tissues, both in unpaired (0.931±1.325 vs 0.018±0.061, \( P < 0.001 \)) and paired samples (1.026±1.559 vs 0.018±0.061, \( P < 0.001 \)) [Fig.1].

High expression of C6orf15 in colon cancer is associated with adverse clinical characteristics

After excluding patients with incomplete clinical data, 454 patients were divided into high and low expression groups according to the expression level of C6orf15. The high expression of C6orf15 was significantly correlated with T stage (\( \chi^2 = 8.30, \ P = 0.04 \)), lymph node metastasis (\( \chi^2 = 36.97, \ P < 0.001 \)), distant metastasis (\( \chi^2 = 8.69, \ P = 0.003 \)), and pathological stage (\( \chi^2 = 34.17, \ P < 0.001 \)) [Table 1].

Table 1 Relationship between C6orf15 expression and clinicopathological factors in colon cancer (N=454)
<table>
<thead>
<tr>
<th>Variables</th>
<th>C6orf15 expression</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>106 (23.3%)</td>
<td>108 (23.8%)</td>
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</tr>
<tr>
<td>Male</td>
<td>121 (26.7%)</td>
<td>119 (26.2%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>&lt;=65</td>
<td>98 (21.6%)</td>
<td>90 (19.8%)</td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>129 (28.4%)</td>
<td>137 (30.2%)</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td>8.30</td>
</tr>
<tr>
<td>T1</td>
<td>10 (2.2%)</td>
<td>1 (0.2%)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>41 (9.1%)</td>
<td>36 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>148 (32.7%)</td>
<td>161 (35.5%)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>27 (6%)</td>
<td>29 (6.4%)</td>
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</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td>36.97</td>
</tr>
<tr>
<td>N0</td>
<td>165 (36.3%)</td>
<td>102 (22.5%)</td>
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</tr>
<tr>
<td>N1</td>
<td>38 (8.4%)</td>
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<td>24 (5.3%)</td>
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<tr>
<td>M stage</td>
<td></td>
<td></td>
<td>8.69</td>
</tr>
<tr>
<td>M0</td>
<td>171 (43.1%)</td>
<td>162 (40.8%)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>20 (5%)</td>
<td>44 (11.1%)</td>
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<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td>34.17</td>
</tr>
<tr>
<td>Stage I</td>
<td>47 (10.6%)</td>
<td>28 (6.3%)</td>
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<tr>
<td>Stage II</td>
<td>108 (24.4%)</td>
<td>68 (15.3%)</td>
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<td>Stage III</td>
<td>45 (10.2%)</td>
<td>83 (18.7%)</td>
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<tr>
<td>Stage IV</td>
<td>20 (4.5%)</td>
<td>44 (9.9%)</td>
<td></td>
</tr>
</tbody>
</table>

Colon cancer patients with higher C6orf15 expression showed a poor prognosis

Kaplan-Meier survival analysis of OS and PFI were performed among 453 patients' data of COAD in TCGA. $p$-value was calculated by log-rank test. The median OS of patients with higher C6orf15
expression was 63.7 months compared to 94.0 months (HR=1.65, \( P=0.013 \)). High expression of C6orf15 also relates to shorter PFI (HR=1.81, \( P=0.001 \)) [Fig.2].

**Gene set enrichment analysis**

GSEA was used to determine the enriched molecular pathways, the results revealed that C6orf15 promotes progression and metastasis of colon cancer by ECM-receptor-interaction, Hedgehog signaling pathway, and Wnt signaling pathway. And C6orf15 negatively regulates some immune-related pathways such as antigen processing and presentation, natural killing cell mediated cytotoxicity and intestinal immune network for IGA production to promote immune escape [Fig.3].

**Immunohistochemical expression of C6orf15 in colon cancer**

A total of 31 patients with primary colon cancer who were diagnosed with primary colon cancer in our hospital from January to February 2022 and received radical surgery were selected and their clinicopathological data were collected. There are respectively 6 patients who staged T3N0M0 and T4N0M0, and 11 of T3 patients had lymph node metastasis. So, these 23 patients were included to perform group analysis. The results showed that the expression of C6orf15 protein in colon cancer tissues was correlated with the depth of invasion (\( P=0.023 \)) and lymph node metastasis (\( P=0.048 \)). (Fig.4)

**Discussion**

With the popularization of colonoscopy and the advancement of early screening, more and more colon cancers can be diagnosed and treated early, but colorectal cancer is still one of the top five cancer morbidity and mortality in the world\([5]\). Some studies have pointed out that with the continuous increase of population aging in China, the burden of cancer is on the rise, and the incidence and mortality of colorectal cancer in my country have increased significantly\([6]\). Therefore, the discovery of new markers which could predict the metastasis, recurrence and prognosis of colon cancer is crucial for the selection of postoperative adjuvant therapy and follow-up monitoring.

The function of C6orf15 has not been clearly elucidated, and previous studies have found that it is expressed in the skin and tonsils\([7]\), which may be related to the susceptibility of follicular lymphoma\([4]\). At the same time, its deletion may be associated with desquamation\([8]\). It is predicted to be involved in extracellular matrix composition, collagen V and fibronectin binding activity\([9]\). The role of this gene in solid tumors has not yet been reported.

Enrichment analysis showed that C6orf15 is mainly involved in the ECM-receptor interaction and the Hedgehog signaling pathway which are associated with tumor shedding, adhesion, and movement, the Hedgehog signaling pathway also plays an important role in avoiding immune destruction\([10,11]\). We speculate that C6orf15 may promote tumor invasion and metastasis by regulating the extracellular matrix and its interaction with neighboring cells.
The results of our study indicate that C6orf15 is up-regulated in colon cancer, and its high expression is associated with advanced stage and worse prognosis. Further speculating on its specific mechanism in colon cancer found that it may be involved in the progression of colon cancer by regulating the extracellular matrix and promoting the Hedgehog signaling pathway related to invasion and metastasis, which is worthy of further experimental exploration.

Methods

Bioinformatic analysis

Transcriptome sequencing data and corresponding clinicopathological data of colon cancer (COAD) and normal colon tissue samples were downloaded from the TCGA database. Wilcoxon rank sum test were used to analyze the differential expression of C6orf15 in tumor tissues and normal tissues. After excluding patients with incomplete clinical data, the patients were divided into high expression and low expression groups according to the median expression of C6orf15, and the correlation between its expression and clinicopathological characteristics such as age, gender, tumor stage (T stage), lymph node metastasis, distant metastasis, and TNM stage were analyzed. Gene set enrichment analysis (GSEA) was performed using the R package clusterProfiler (version 3.14.3). The annotated gene set (c2.all.v 7.2. symbols. gmt) was selected as the reference gene set, and the number of calculations was set to 1000 times, satisfying p.adj<0.05 and FDR<0.25 are significantly enriched gene sets.

Survival analysis

Overall survival (OS) and Progression-free interval (PFI) were used for survival analysis. The effect of C6orf15 expression on the prognosis of patients was assessed by the log-rank test and the survival curve was drawn using the Kaplan-Meier method.

Clinical tissue sample collection

To validate the predictive role of C6orf15, we collected the colon cancer samples confirmed by hematoxylin-eosin staining from 2022.01 to 2022.02 in the department of Gastroenterological Surgery of Peking University People's Hospital. Patients who were taken other treatments before surgery were excluded, a total of 31 colon cancer tissue samples were taken for immunohistochemical staining.

Immunohistochemistry

Colon cancer tissue samples were fixed by 4% paraformaldehyde (pH 7.4), embedded in paraffin, serially sectioned at 5 µm, and rehydrated through a series of alcohol and water baths. The tissue sections were placed in a repair box filled with citric acid (PH 6.0) antigen retrieval buffer and heated to boil for antigen retrieval. Then, the sections were placed in phosphate-buffered saline (PBS, PH7.4) to cool to room temperature. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide at room temperature for 15 min. Then the slides were blocked with 3% BSA for 30 min at room temperature. The selected slides were immune-stained with a C6orf15 polyclonal antibody (proteintech, 24953-1-AP, 1:600)
as the primary antibody through incubation in a wet box overnight at 4°C. Detection of primary antibodies was performed using a commercial envision detection kit containing the secondary antibody (Dako, GK500705). Subsequently, the slides were incubated in prepared 3,3-diaminobenzidine from the kit at room temperature for 10 min. Slides were then counterstained with hematoxylin and dehydrated through a series of ethanol and xylene baths. Expression of the stained markers was scored using a histologic score (H-score)\textsuperscript{[12]}.

**Statistical analysis**

R (v.3.6.3) and GraphPad Prism (8.0) were used for data analysis. All gene expression data were log\textsubscript{2} transformed. The Wilcoxon rank sum test was used to compare the expression of C6orf15 in tumor tissue and normal tissue, and the \( \chi^2 \) test was used to analyze the correlation between C6orf15 mRNA expression and clinicopathological indicators. T-test was used to compare the relationship between the protein expression and the clinicopathological characteristics in different groups. \( P<0.05 \) was regarded as a statistically significant difference.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Research Ethics Committee of the Peking University People's Hospital (2021PHD010-001).

**Availability of data and materials**

The raw data of this study are derived from the TCGA database (https://portal.gdc.cancer.gov/), which are publicly available database.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

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**Authors’ contributions**

This research was conducted in collaboration with all authors. XX and SW performed the data analysis. XX and ZG analyzed and interpreted the results. XX, YY and ZG drafted and reviewed the manuscript. All authors read and approved the final manuscript.

**References**


Figures
Figure 1

The expression of C6orf15 in colon cancer and normal tissues. (A) The expression of C6orf15 mRNA was higher in tumors than in normal tissues; (B) The expression levels of C6orf15 in tumors and paired normal tissues in 41 colon cancer patients. ***$P<0.001$.

Figure 2

Kaplan-Meier survival analysis of OS and PFI among 453 patients of colon cancer in TCGA database.
Enrichment analysis identified several cancer-related and immune related KEGG pathways. (A-C) Elevated C6orf15 is positively correlated with various cancer-related pathways. (D-F) Elevated C6orf15 is negatively correlated with some immune-related pathways.
Figure 4

Comparison of C6orf15 expression in different histology groups ×100. (A-B) Higher expression of C6orf15 is associated with deeper infiltration depths; (C-D) Higher expression of C6orf15 is associated with lymph nodes metastasis in T3 stage patients.