Functional genetic variant of HSD17B12 in the fatty acid biosynthesis pathway predicts the outcome of colorectal cancer

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Abstract
Fatty acids are involved in the development and progression of colorectal cancer (CRC). However, genetic effects of fatty acid biosynthesis pathway on CRC outcome are unclear. Cox regression model was used to evaluate genetic effects on CRC overall survival (OS) and progression-free survival (PFS), accompanied by calculating hazard ratios (HRs) and confidence intervals (CIs). Differential expression analysis, expression quantitative trait loci analysis, dual-luciferase reporter assay and chromatin immunoprecipitation assay were performed to explore the genetically biological mechanism. The rs10838164 C>T in HSD17B12 was significantly associated with an increased risk of death and progression of CRC (OS, HR = 2.12, 95% CI = 1.40-3.22, P = 4.03 × 10⁻⁴; PFS, HR = 1.64, 95% CI = 1.11-2.44, P = 1.35 × 10⁻²), of which T allele could increase HSD17B12 expression (P = 1.78 × 10⁻¹¹). Subsequently, the functional experiments indicated that rs10838164 T allele could not only enhance the binding affinity of transcription factor YY1 to HSD17B12 region harbouring rs10838164 but also promote the transcriptional activity of HSD17B12, which was significantly up-regulated in colorectal tumour tissues. Our findings suggest that genetic variants in fatty acid biosynthesis pathway play an important role in CRC outcome.
1 | INTRODUCTION

Colorectal cancer (CRC) ranks the fourth in cancer incidence and the second in cancer-related mortality globally, causing serious harm to human health. In the past decades, great advancements in diagnosis and treatment strategies have been achieved. However, the incidence and morbidity of CRC remain unfavourable in China. Accumulating evidence suggests that environmental, genetic and epigenetic factors all contribute to the development and progression of CRC. It is well known that although the patients with CRC have the same clinicopathologic characteristics and undergo similar therapeutic regimens, the overall survival (OS) still varies from person to person, indicating that genetic factors play an essential role in the variable survival.

Fatty acids, important sources of energy, promote the proliferation of cancer cells by synthesizing cellular membranes and signaling molecules. The building blocks of fatty acids are mainly derived from exogenous lipids or de novo fatty acid synthesis. Normal cells usually obtain fatty acids in an exogenous dependent manner. While tumour cells are dependent on fatty acids de novo. Several studies have confirmed that fatty acids are closely associated with tumour growth, invasion, metastasis and drug resistance. Additionally, previous studies suggested that turbulence of biosynthesis and metabolism of fatty acids are involved in the development and recurrence of cancers, including CRC, lung cancer, and prostate cancer.

Single-nucleotide polymorphisms (SNPs) are associated with the risk and survival of cancer. The correlation between SNPs in fatty acid biosynthesis pathway genes and cancer risk has been reported in CRC, prostate cancer, and lung cancer. Moreover, genetic variants in fatty acid biosynthesis are also linked to cancer survival in malignant melanoma. However, the relationship between genetic variants in fatty acid biosynthesis pathway genes and CRC survival is still unknown.

In this study, we hypothesized that the functional genetic variants in the fatty acid biosynthesis pathway were associated with CRC survival. Herein, we assessed the genetic effect of SNPs in the fatty acid biosynthesis pathway on CRC survival in a Chinese population and investigated the relevant genetic functions.

2 | MATERIALS AND METHODS

2.1 | Study participants

In this study, 344 patients were recruited from the First Affiliated Hospital of Nanjing Medical University and Nanjing First Hospital. All participants were histopathologically diagnosed with CRC and unrelated Han Chinese. Meanwhile, 5-mL blood samples were collected from each patient. Clinical and survival information was collected from medical records and telephone interviews, respectively. The recruitment and follow-up of population information were started from September 2010, and the deadline for follow-up data was April 2, 2016. For all participants, the OS information was missing in 57 patients. OS as the primary endpoint was calculated from the date of CRC diagnosis to the date of cancer-related death or the last follow-up. Progression-free survival (PFS) as the second endpoint was calculated from the date of CRC diagnosis to the date of primary metastasis. The written informed consent was provided by all participants, and the study was approved by the Institutional Review Board of Nanjing Medical University.

2.2 | Selection of gene and SNP

Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/pathway.html), UniProt (https://www.uniprot.org/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed) were used to screen key genes in the fatty acid biosynthesis pathway. We included 31 candidate key genes for further analysis (Table S1 and Figure S1).

For each candidate gene, chromosomal positions were determined in the UCSC Genome Browser (GRCH 37; https://genome.ucsc.edhifu). SNPs located in these genes and the corresponding 2 kb upstream and downstream regions were extracted for association analysis. First, the genotype data were extracted from the 1000 Genomes Project in the populations of Beijing, China (CHB), and Tokyo, Japan (JPT). Quality control was based on three criteria: (a) minor allele frequency (MAF) ≥ 0.05; (b) Hardy-Weinberg equilibrium (HWE) of P value ≥ 0.05; and (c) genotyping call rate >95%. Second, PancanQTL (http://bioinfo.life.hust.edu.cn/PancanQTL/), RegulomeDB (http://regulome.stanford.edu/) and HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) were used to select potentially functional SNPs. As shown in Table S2, SNPs were retained with the expression quantitative trait loci (eQTL) function in colon adenocarcinoma or rectal adenocarcinoma from PancanQTL and a score of <6 from RegulomeDB. Lastly, tagging SNPs were recognized using pairwise linkage disequilibrium (LD) analysis (r² ≥ 0.8) via the HaploView 4.2 software.

2.3 | SNP genotyping

The details of genotyping are available in previous studies. Briefly, the genomic DNA was extracted from the peripheral
venous blood samples using the Qiagen Blood Kit (Qiagen) according to the manufacturer’s instructions. DNA was successfully extracted from the samples collected from 344 patients. Genotyping was performed with Illumina Human Omni Zhong Hua Bead Chips. Genotype analysis was independently conducted by two individuals. We followed a uniform quality control protocol to filter the samples and SNPs.

2.4 | Gene expression and eQTL analysis

We downloaded mRNA expression data of candidate genes on CRC and normal tissues from The Cancer Genome Atlas (TCGA) database (log₂ transformed), comprising of 625 CRC tissues and 51 normal tissues, and the Gene Expression Omnibus (GEO) datasets (GSE21510, GSE74602, GSE113513, and GSE87211). The relationship between the expression of candidate genes and survival of CRC was also analysed by extracting the data from TCGA database and merged them with the corresponding expression data from TCGA database. The GEPIA (http://gepia.cancer-pku.cn/) online tool, including TCGA database, was used to assess the expression pattern of candidate genes in pan-cancer. The Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) was used to evaluate the protein expression of the candidate genes. To further confirm the genetic effects of significant SNPs on the mRNA expression levels, we obtained the access to download the genotype data from TCGA database and merged them with the corresponding expression of mRNA.

2.5 | Luciferase reporter assays

The 1000-bp region surrounding the rs10838164 C or T alleles and HSD17B12 promoter region were synthesized and inserted into the pGL3-basic vector (Promega) by two restriction sites (KpnI and NheI). The synthesized plasmids and Renilla plasmids were co-transfected into CRC cell lines (DLD-1 and HT29) were seeded into 24-well plates with a density of 3 × 10⁵ cells/well) using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s protocols. After 24 hours of incubation, Firefly and Renilla luciferase activities were measured using the dual-luciferase kit (GeneCopoeia). Relative luciferase activity was normalized to the Renilla luciferase.

2.6 | Transfection

DLD-1 and HT29 cells were seeded into a six-well plate at 50% confluence and transfected with specific siRNA targeting YY1, YY1 overexpressed plasmids, and corresponding control plasmids (GenePharma) using Lipofectamine 3000 (Invitrogen). After 48 hours of transfection, we extracted RNA and protein to detect the knockdown or overexpression efficiency.

2.7 | RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from CRC cells by using TRizol reagent (Invitrogen). The cDNA was synthesized by using Primescript RT Reagent (Takara), according to the manufacturer's instructions. Quantitative real-time PCR (RT-qPCR) was carried out on the StepOnePlus Real-Time PCR system (Applied Biosystems) with the SYBR Green reagents (Takara). The relative expression was calculated by the 2⁻ΔΔCt method and normalized to β-actin. All PCR primer sequences used in this study are listed in Table S3.

2.8 | Protein isolation and Western blot

Total cellular proteins were isolated by incubating the cells with RIPA buffer containing 1% PMSF (Sigma) on ice for 30 minutes. The protein concentrations were quantified by BCA Protein Assay Kit (Beyotime). Proteins were electrophoresed by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). The membranes were then blocked with 5% skimmed milk for 2 hours at room temperature. Subsequently, membranes were incubated with the primary antibodies, including anti-YY1 (63227, Cell Signaling Technology 1:1000) and anti-β-actin (3700, Cell Signaling Technology 1:1000) overnight at 4°C. Next morning, the membrane was incubated with peroxidase (HRP)-conjugated secondary antibody (Proteintech, 1:5000) for 1 hour at room temperature. Finally, the intensity of the bands was detected by the chemiluminescence system (Bio-Rad) and analysed by Image Lab software.

2.9 | Chromatin immunoprecipitation (ChIP) assay

We performed ChIP experiments in DLD-1 cells using a CHIP-IT Express Enzymatic kit (53009, Active motif). The assay was conducted in four steps with a sequence as the following: crosslinking, fragmentation, immunoprecipitation and purification. Briefly, cells were crosslinked with 1% formaldehyde for 10 minutes. Then, the working stock of enzymatic shearing cocktail was added and incubated at 37°C for 15 minutes to shear chromatin. The sheared chromatin was incubated overnight with protein G magnetic beads and YY1 antibodies (63227, Cell Signaling Technology), or normal rabbit IgG as a negative control. FAM193B, a known target gene of YY1, was selected as the positive control according to the description of YY1 antibodies (63227, Cell Signaling Technology). DNA was purified using the Chromatin IP DNA Purification kit (58002, Active motif). Finally, RT-qPCR was performed with specific primers for the YY1 binding site (Table S3).

2.10 | Statistical analysis

Cox regression analysis, including unconditional univariate and multivariate analyses, was conducted to assess the association of the
candidate SNPs and CRC outcomes (OS and PFS). Hazard ratios (HRs) and its corresponding 95% confidence intervals (CIs) were calculated to evaluate the genetic effects. To reduce the possibility of false positive findings, we used the false discovery rate (FDR) approach for multiple comparisons. Student’s t test was used to compare differences in gene expression between CRC and normal tissues. The Kaplan-Meier curve method was used to assess the association between survival time and the expression levels of gene mRNA. Statistical analyses were performed with R software (Version 3.2.5), PLINK (Version 1.09) and Review Manager Software (Version 5.3). For all statistical analyses, a two-sided P value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Association between SNPs in the fatty acid biosynthesis pathway and CRC survival

As shown in Figure 1, a total of 23 index SNPs in the fatty acid biosynthesis pathway genes were selected for further association analysis after SNP screening and functional annotation. We evaluated the genetic effects of 23 SNPs on the OS of patients with CRC in the additive genetic model (Table S4). Only rs10838164 in HSD17B12 was found to be associated with OS in patients with CRC after FDR correction (\( HR = 2.12, 95\% CI = 1.40-3.22, P = 4.03 \times 10^{-4}, P_{FDR} = 9.27 \times 10^{-3} \)).

Next, we dissected the genetic effects of HSD17B12 rs10838164 on CRC prognosis in different genetic models (Table 1). In the additive genetic model, individuals with T allele were also notably related to an increased risk of progression compared with those with C allele (\( HR = 1.64, 95\% CI = 1.11-2.44, P = 1.35 \times 10^{-2} \) for PFS). A similar genetic effect was found in the dominant genetic model under survival and progression (\( HR = 2.30, 95\% CI = 1.45-3.65, P = 4.10 \times 10^{-4} \) for OS; \( HR = 1.82, 95\% CI = 1.17-2.83, P = 7.97 \times 10^{-3} \) for PFS; Figure 2).

3.2 | Stratified analysis for the association between HSD17B12 rs10838164 and CRC outcome

Stratified by demographics (Figure 3A), we found a significant genetic effect of HSD17B12 rs10838164 on OS in non-smokers (\( HR = 2.96, 95\% CI = 1.67-5.26, P = 2.09 \times 10^{-4} \)), non-drinkers (\( HR = 2.28, 95\% CI = 1.31-3.95, P = 3.46 \times 10^{-3} \)) and no family history (\( HR = 2.45, 95\% CI = 1.48-4.04, P = 4.90 \times 10^{-3} \)). In the subgroup of clinical characteristics (Figure 3A), a significant association was found in the patients with well + moderate (\( HR = 2.53, 95\% CI = 1.40-4.58, P = 2.12 \times 10^{-3} \)) and Dukes C + D (\( HR = 2.35, 95\% CI = 1.43-3.87, P = 7.51 \times 10^{-4} \)). Additionally, for the association between HSD17B12 rs10838164 and PFS (Figure 3B), we observed a significant decreased PFS in the patients aged ≤60 years (\( HR = 2.24, 95\% CI = 1.11-4.49, P = 2.37 \times 10^{-2} \)), male (\( HR = 1.79, 95\% CI = 1.02-3.14, P = 4.10 \times 10^{-2} \)), non-smokers (\( HR = 1.97, 95\% CI = 1.17-3.33, P = 1.13 \times 10^{-2} \)) and the patients with Dukes C + D (\( HR = 1.86, 95\% CI = 1.16-2.99, P = 1.06 \times 10^{-3} \)). However, no heterogeneity existed in each subgroup.

3.3 | SNP rs10838164 allelic-specific impact on transcriptional activity of HSD17B12

To explore the effect of rs10838164 on HSD17B12 expression, we conducted eQTL analysis and found that the rs10838164 C>T could significantly increase the expression levels of HSD17B12 in tumours (\( P = 1.78 \times 10^{-11} \); Figure 4A). Considering the notable eQTL and survival effects of rs10838164, we hypothesized that rs10838164 could regulate the expression of HSD17B12 as a promoter-like function in CRC. To verify the hypothesis, we conducted luciferase report assays and observed that the luciferase activity of the region containing the rs10838164 T allele was notably higher than that of the region containing C allele in both DLD-1 and HT29 cell lines (Figure 4B-D). It suggested that the T allele could enhance the transcriptional activity of HSD17B12 in CRC.

3.4 | Effect of rs10838164 on transcriptional activity is mediated by YY1

Previous studies revealed that the SNP in the non-coding region might regulate gene expression by altering the binding affinity of the transcription factors. As rs10838164 is located in the intron of HSD17B12 and based on the above-mentioned findings, we hypothesized
## Table 1 Association of HSD17B12 rs10838164 with OS and PFS in CRC patients

| Endpoints                        | Genetic models | Genotyping | No. events (%) | Univariate analysis | HR (95% CI) | P       | HR (95% CI) | P       |
|----------------------------------|----------------|------------|----------------|---------------------|-------------|---------|-------------|---------|
|                                  |                |            |                |                     |             |         |             |         |
|                                  | CC             | 251        | 131 (43.09)    |                     | 1.00        |         | 1.00        |         |
|                                  | CT             | 28         | 21 (67.74)     |                     | 2.29 (1.43-3.65) | 5.24×10⁻⁴ | 2.27 (1.42-3.63) | 6.37×10⁻⁴ |
|                                  | TT             | 1          | 1 (100.00)     |                     | 2.57 (0.36-18.51) | 3.48×10⁻¹ | 3.29 (0.44-24.85) | 2.49×10⁻¹ |
|                                  | Missing        | 7          |                |                     |             |         |             |         |
|                                  | Additive model |           |                |                     | 2.11 (1.39-3.20) | 4.13×10⁻⁴ | 2.12 (1.40-3.22) | 4.03×10⁻⁴ |
|                                  | Dominant model |           |                |                     | 2.30 (1.45-3.63) | 3.77×10⁻⁴ | 2.30 (1.45-3.65) | 4.10×10⁻⁴ |
|                                  | Recessive model|           |                |                     | 2.32 (0.32-16.66) | 4.02×10⁻¹ | 2.92 (0.39-22.03) | 2.98×10⁻¹ |
|                                  |                |            |                |                     |             |         |             |         |
|                                  | CC             | 289        | 218 (71.71)    |                     | 1.00        |         | 1.00        |         |
|                                  | CT             | 28         | 22 (70.97)     |                     | 1.82 (1.16-2.83) | 8.61×10⁻³ | 1.81 (1.15-2.83) | 1.01×10⁻² |
|                                  | TT             | 1          | 1 (100.00)     |                     | 1.37 (0.19-9.76) | 7.57×10⁻¹ | 2.20 (0.30-16.37) | 4.40×10⁻¹ |
|                                  | Missing        | 8          |                |                     |             |         |             |         |
|                                  | Additive model |           |                |                     | 1.64 (1.11-2.43) | 1.32×10⁻² | 1.64 (1.11-2.44) | 1.35×10⁻² |
|                                  | Dominant model |           |                |                     | 1.79 (1.16-2.77) | 8.91×10⁻³ | 1.82 (1.17-2.83) | 7.97×10⁻³ |
|                                  | Recessive model|           |                |                     | 1.30 (0.18-9.27)  | 7.95×10⁻¹ | 2.05 (0.28-15.16) | 4.48×10⁻¹ |

Abbreviations: CI confidence interval; CRC, colorectal cancer; HR hazards ratio; OS, overall survival; PFS, progression-free survival.

*Adjusted for age, sex and Dukes stage in Cox regression model.

*Genotyping for patients with corresponding endpoints (OS or PFS).
that rs10838164 C>T could enhance the transcriptional activity of HSD17B12 by affecting the binding affinity of the transcription factor to HSD17B12. By bioinformatics analysis (PROMO and JSAPAR), we found that the region surrounding rs10838164 was enriched with transcription factor Yin Yang-1 (YY1) binding sites, which indicated that rs10838164 T allele might increase HSD17B12 expression by regulating YY1 binding affinity (Figure S2). To confirm this hypothesis, we transfected YY1 siRNA or overexpressed plasmids into DLD-1 and HT29 cells, of which siRNA2 with the highest interference efficiency was selected for further study (Figure S3). Importantly, the different luciferase activity between the rs10838164 C allele and T allele was diminished after the YY1 deletion, and both alleles could inhibit the activity levels compared with the HSD17B12 promoter. In contrast, the rs10838164 C allele and T allele both promoted the activity levels after YY1 overexpression (Figure 4C-D). Meanwhile, we also performed RT-qPCR assays to evaluate the HSD17B12 mRNA expression levels. As expected, knockdown of YY1 remarkably decreased the HSD17B12 mRNA expression levels, while overexpression of YY1 increased the HSD17B12 mRNA expression levels (Figure 4E-H). In the TCGA and GEO databases, the expression level of YY1 to the region containing HSD17B12 rs10838164 in DLD-1 cells. As shown in Figure 4I, our results confirmed that YY1 preferentially bound to the region harbouring T allele using ChIP followed by allele-specific RT-qPCR. Taken together, these findings supported that rs10838164 could regulate the expression of HSD17B12 by affecting the binding of YY1 to HSD17B12.

3.5 | HSD17B12 expression pattern in CRC tissues

In the TCGA database, HSD17B12 was highly expressed in colorectal tumour tissues compared with that in normal tissues ($P = 6.00 \times 10^{-4}$ in unpaired tissues and $P = 4.66 \times 10^{-2}$ in paired tissues; Figure 5A-B). In the HPA database, the protein expression of HSD17B12 was also higher in CRC tissues than that in normal tissues (Figure 5). Consistently, this same expression pattern was further validated in GEO datasets (Figure 5D-F). Additionally, the expression of the other key genes in fatty acid biosynthesis in TCGA and GEO databases were presented in Figure S6. However, we found no significant association between the expression of HSD17B12 and OS in patients with CRC (Figure S7).
In this study, we investigated the association between SNPs in the fatty acid biosynthesis pathway and CRC outcome. Our findings suggested that HSD17B12 rs10838164 was significantly related to the survival of CRC in the Chinese population. SNP rs10838164 showed a gene regulatory role in the expression of HSD17B12 by affecting the binding affinity of YY1 in CRC. Moreover, HSD17B12 was highly expressed in tumour tissues compared with the adjacent normal tissues from the databases. Therefore, we speculated that rs10838164 might affect the function of HSD17B12 in CRC by regulating the gene expression levels.

The disorder of metabolism, including the metabolism of fatty acids, has been considered to be one of the important characteristics of malignant tumours, which confers cancer cells the ability to survive, proliferate and metastasize. Previous studies suggested that fatty acid biosynthesis was significantly up-regulated in tumour cells to provide energy for the synthesis of signalling molecules and membranes. Additionally, the correlation between fatty acid biosynthesis and tumours has been demonstrated in many
aspects, including risk, survival, recurrence and drug resistance. For example, the expression of ACY is higher in various types of tumour tissues than that in adjacent normal tissues. FASN is also up-regulated in cancer tissues and promotes invasion and metastasis of CRC. Meanwhile, the overexpression of FASN is linked to poor survival and drug resistance in breast, prostate and bladder cancers. Furthermore, blocking or deregulating fatty acid biosynthesis can attenuate cancer proliferation, metastasis and recurrence in cancers, including CRC. All these studies indicate that fatty acid biosynthesis plays a necessary role in tumours.

Recently, the relationship of genetic variants in fatty acid biosynthesis and cancer has been evaluated. For example, genetic polymorphisms in fatty acid biosynthesis are associated with survival in patients with hepatocellular carcinoma and cutaneous melanoma. However, the association between the genetic variant of fatty acid biosynthesis and the survival of patients with CRC has not been well studied.

HSD17B12, located at 11p11.2, acts as a multifunctional isoenzyme for the extension of long-chain fatty acids and the conversion of oestrone to oestradiol, in particular the conversion of palmitic to arachidonic acid. HSD17B12 has been identified as an oncogene in various cancers. Studies have shown that HSD17B12 mRNA expression level is higher in breast tumour tissues than in normal tissues and negatively correlated with prognosis. Moreover, the patients with the overexpression of HSD17B12 have worse OS and PFS than those with low expression in ovarian cancer. Consistently, our findings showed that HSD17B12 was overexpressed in CRC tissues compared with normal tissues. Furthermore, the expression of HSD17B12 in CRC tissues was the highest among the pan-cancer tissues, which indicated that HSD17B12 might play an essential role in CRC. We also found a trend in adverse prognosis between HSD17B2 expression and CRC although it was not statistically significant. This result may be caused by the analysis that was mostly based on the European population. Therefore, the association between the HSD17B2 mRNA expression and OS of CRC needs to be explored in the Chinese population.

Genetic variants in HSD17B2 have been investigated in several cancers. HSD17B2 rs11037684 A>G is associated with poor...
OS in patients with cutaneous melanoma. Genetic variants in HSD17B12 are associated with a high risk of recurrence in prostate cancer. Moreover, HSD17B12 rs7932905 is associated with prostate aggressiveness at the time of diagnosis. However, no reports have shown the relationship between genetic variants in HSD17B12 and CRC. Our study is the first to reveal the significant association between HSD17B12 rs10838164 and outcomes of CRC in the Chinese population. Moreover, stratification analysis showed that rs10838164 T allele predicted worse OS and PFS in patients with Dukes C + D than in patients with Dukes A + B. The eQTL analysis showed that rs10838164 was associated with HSD17B12 expression and reporter assays indicated that T allele of rs10838164 could increase the expression of HSD17B12 by altering its transcriptional activity.

Previous studies have shown that the SNP in the non-coding region might regulate gene expression via altering RNA splicing, RNA degradation and transcription factor binding affinity. The rs10838164 is located in the intron of HSD17B12. We observed that the region surrounding rs10838164 was enriched with the binding sites of YY1, which was selected to evaluate the effect of rs10838164 on HSD17B12 expression. YY1 is a ubiquitous zinc finger transcription factor that can act as a transcriptional activator or repressor, depending upon the interacting partners. Numerous studies have shown that YY1 is highly expressed in various tumours and involved in tumour progression by targeting different genes. As reported by Wang et al., YY1 stimulates tumorigenesis and the Warburg effect by up-regulating GLUT3 expression in CRC cells. Zhu et al. found that YY1 activates the SLC22A15 and AANAT expression to promote the proliferation of CRC cells. Furthermore, YY1 can also suppress fatty acid oxidation by targeting PGC-1α and thus lead to lipid accumulation in liver cancer cells. We observed that knockdown of YY1 abrogated the different effects of the rs10838164 C allele and T allele on the transcriptional activity of HSD17B12. Additionally, rs10838164 C>T enhanced the binding affinity of YY1 to HSD17B2. The HSD17B2 mRNA expression levels changed in the same direction with YY1 knockdown or over-expression in CRC cells. Taken together, our findings suggested that rs10838164 C>T could modulate the transcriptional activity of HSD17B2 by influencing the binding affinity of YY1 and thus altering the HSD17B2 expression level.

There are several limitations to this study. First, the information about treatments, a factor with the potential effects on CRC survival, is not applicable, which should be adjusted for survival assessment. Second, the number of participants recruited in this study was not large enough to perform stratified analysis in a recessive genetic model. Thus, larger populations are warranted to confirm our findings. Third, we did not validate the relationship between HSD17B2 expression and SNP rs10838164 in the in-house clinical samples. Finally, additional functional investigations should be conducted to clarify the function and underlying molecular mechanism of HSD17B2 on the survival of CRC.

In conclusion, our present study investigated the association between genetic variants in fatty acid biosynthesis genes and CRC survival in the Chinese population. We identified a novel genetic variant, HSD17B12 rs10838164 C>T, which was significantly linked to worse OS and PFS in patients with CRC. The rs10838164 C>T regulated HSD17B12 expression by altering the YY1 binding affinity, which might be a possible mechanism affecting the outcome of CRC patients. Moreover, an up-regulated expression of HSD17B2 was observed in CRC tissues in both TCGA and GEO databases. Our findings suggest that HSD17B12 rs10838164 is associated with outcome of CRC, which might be a potential prognostic marker for patients with CRC.

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CONFLICT OF INTEREST

The authors indicate no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yu Lin: Data curation (equal); formal analysis (equal); investigation (lead); validation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Yixuan Meng: Data curation (equal); formal analysis (equal); investigation (supporting); validation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Jinying Zhang: Data curation (equal); resources (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Ling Ma: Formal analysis (supporting); project administration (supporting); supervision (supporting); writing-review & editing (supporting). Lu Jiang: Formal analysis (supporting); methodology (supporting); resources (supporting); writing-original draft (supporting). Yi Zhang: Formal analysis (supporting); resources (supporting). Ming Yuan: Resources (supporting); visualization (supporting). Anjing Ren: Methodology (supporting); supervision (supporting). Weiyou Zhu: Resources (supporting); validation (supporting). Shuwei Li: Methodology (supporting); supervision (supporting); writing-review & editing (supporting). Yongqian Shu: Resources (supporting); supervision (supporting). Linji Zhang: Conceptualization (equal); funding acquisition (supporting); methodology (equal); supervision (equal); writing-review & editing (equal). Lingqian Zhu: Conceptualization (equal); funding acquisition (lead); methodology (equal); project administration (lead); resources (equal); supervision (equal); writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

The data are available upon reasonable requests.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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