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Mendelian Randomization Analyses of Circulating Levels of Insulin-Like Growth Factor 1, Genetic Polymorphism, and Their Associations with Colorectal Cancer

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SZ and XL conducted the experiments in this study. HW and JH contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
Insulin-like growth factor 1, Colorectal cancer, Genetic polymorphism, Circulating level, Mendelian, Randomization

ABSTRACT

Insulin-like growth factor 1 (IGF-1) may exert a crucial role in the progression of colorectal cancer (CRC). This work aimed to investigate the Mendelian randomization (MR) relationship between circulating level (CL) of serum IGF-1, genetic polymorphism, and their associations with CRC. Two hundred eighty-three patients with CRC at our hospital who were diagnosed during January 2019 and December 2022 were collected and marked as a cancer group (CCG). On the other hand, three hundred twenty healthy patients who underwent routine physical examinations at our hospital during the same period were selected as a control group (CTG). Furthermore, genotypes of three IGF-1 genes, rs6218, rs35767, and rs574261, were analyzed using MR analysis. In addition, association between CL of serum IGF-1 and CRC was also examined. IGF-1 rs6218 (P=0.004), rs574261 (P=0.006), and rs574261 (P=0.008) showed obvious correlations with the risk of CRC. Through a MR analysis of rs6218 and rs35767, a mutation in these genes led to a respective 1.43% (P=0.039) and 1.28% (P=0.046) increase in the risk of CRC for each additional risk allele (ARA) of IGF-1. This work revealed a close relationship between elevated IGF-1 and increased risk of CRC, and mutation of genetic polymorphism in IGF-1 may serve as a predictive marker for occurrence of CRC. These findings offered an essential groundwork for screening high-risk populations and developing personalized preventive and therapeutic approaches for CRC.

INTRODUCTION

Colorectal cancer (CRC) is a prevalent malignancy originating from the colon or rectal tissues (Munir, 2020). Among the "top five" cancers, which include gastric cancer, lung cancer, breast cancer, and liver cancer, CRC, it accounts for approximately 9.2% of global mortality. The 5-years survival rate for CRC patients is reported to be only 65%, and CRC exhibits a higher incidence in males than females (Dekker *et al*., 2019; Zhang and Yin, 2020). Recently, incidence of CRC has been on the rise among individuals below middle age (Siegel *et al*., 2017). Regular CRC screening can facilitate the early detection and removal of precancerous adenomas, thereby reducing the incidence and mortality of CRC (Wolf *et al*., 2018). However, factors such as chronic stress, alterations in gut microbiota, and Westernized dietary habits can still contribute to the development of early-onset CRC (Hofseth *et al*., 2020). Research has indicated that 10-20% of CRC patients have a familial hereditary predisposition to CRC, and around 5% of CRC cases are associated with known hereditary CRC syndromes detectable through pedigree analysis. Additionally, an increased incidence of sporadic CRC has been linked to long-term inflammatory bowel disease (Li *et al*., 2021). Currently, clinical CRC screening primarily involves fecal testing, colonoscopy, and tumor marker detection. However, due to the complexity and diversity of CRC, existing diagnostic methods still have certain limitations.

Insulin-like growth factor 1 (IGF-1) exerts a crucial effect in various cellular processes such as cell growth, division, and differentiation, as well as exhibiting anti-

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apoptotic properties (Kasprzak, 2023). IGF-1 binds to insulin-like growth factor binding proteins (IGFBPs) to activate downstream signaling pathways (SPWs), regulating cellular behaviors. IGF-1 is primarily regulated by growth hormone. Under normal circumstances, circulating level (CL) of IGF-1 remains within a relatively stable range. However, multiple reports have explored the association between CL of IGF-1 and genetic polymorphism and the risk of CRC. Some studies suggest that downregulation of IGF-1 and its downstream targets is a key mechanism of the anticancer effects of exercise. In male Wistar rats, an 8-week exercise regimen led to downregulation of IGF-1, proliferating cell nuclear antigen (PCNA), and P-Erk1/2 expression, while IGFBP-3 expression was upregulated. Exercise increased the number of goblet cells and improved colonic structure, indicating the involvement of the IGF-1/IGFBP-3/Erk1/2 SPW in CRC (Darband *et al*., 2021). Studies on populations have confirmed the connection between obesity and the mechanisms underlying CRC, revealing that IGF-1 levels increase in obese individuals (Duraiyarasan *et al*., 2022). This elevation can stimulate the IGF-1 / homeobox A13 (HOXA13) / ATP citrate lyase (ACLY) SPW, inducing colon cell proliferation and promoting CRC initiation and progression (Qiao *et al*., 2021). Studies have also linked IGF-1 to ulcerative colitis (UC). Investigation into the susceptibility relationship of rs6214 (C > T) in IGF-6214 in the Chinese Han population revealed that rs6214 enhances miR-2053 binding, increasing UC susceptibility and inhibiting IGF-1 expression (Wang *et al*., 2023). Additionally, a study by Li *et al*. (2022) found a significant correlation between IGF1 rs35767 (A>G) polymorphism and CRC staging, with CRC patients possessing the rs35767A allele being more likely to have advanced tumor stages. Literature has reported the association between rs2195239 polymorphism under IGF-1 and risk of gastric cancer in the Chinese Han population (Meisami and Jalilvand, 2020). The genetic polymorphism of IGF-1 likely regulates the CRC susceptibility. Genetic polymorphism refers to the existence of multiple different allele forms of a gene, which may affect gene expression or function.

The correlation between CL of serum IGF-1 and genetic polymorphism with CRC has garnered widespread attention and research interest. Understanding association between genetic polymorphism in IGF-1 promoter region tagSNPs and the occurrence and development of CRC can offer a theoretical foundation for early diagnosis, prevention, and treatment by timely intervention in SNPs that may elevate CRC risk. However, understanding on association between IGF-1 and CRC remains limited, and further research is needed to validate this relationship. The aim of this study is to utilize the Mendelian randomization (MR) method to explore the connection between CL of serum IGF-1 and genetic polymorphism with CRC, offering new insights and strategies for preventing and treating CRC.

MATERIALS AND METHODS

Subjects

In this work, two hundred eighty-three patients with CRC at our hospital from January 2019 to December 2022 were enrolled as a cancer group (CCG). Additionally, a control group (CTG) consisted of three hundred twenty individuals who underwent routine physical examinations at our hospital during the same time frame. All cases enrolled belonged to the same geographical area and ethnicity, and all participants were informed about and provided consent by signing an informed consent form for this study. The CTG consisted of individuals who underwent medical examinations at our hospital and did not have underlying metabolic disorders or other tumors. They also did not have cardiovascular diseases. In contrast, the CCG comprised patients diagnosed with CRC at our hospital.

Collection of specimens

In the early morning, 5 mL of fasting peripheral blood was obtained from patients in the CCG and individuals in the CTG into tubes containing heparin and stored at -80 °C. Clinical information was collected from patients in the CCG, including gender, age, tumor location, TNM pathological stage, and cancer cell differentiation level.

DNA extraction and genetic typing

The plasma of patients was subjected to a 15-min centrifugation at 2,500 rpm. The collected white blood cells (WBCs) were transferred to a new centrifuge tube. Subsequently, 3 times the volume of red blood cell lysis buffer (Product No.: R1010, Brand: Solarbio) was added to the remaining plasma. The mixture was placed in an icebox for 15 min and gently shaken on a shaker. Subsequently, centrifugation was performed at 2,500 rpm for 10 min for discarding the supernatant, and an appropriate amount of phosphate-buffered saline (PBS) was added to the precipitate. Another centrifugation step was implemented at 2,500 rpm for 6 min. After supernatant discarding again, the samples were stored at -80 °C.

Using the collected WBCs as the material, DNA extract was implemented using the QIAamp DNA Micro Kit (50) reagent kit (Product No.: 56304). The procedure was as follows: Buffer GA (400 µL) was added to the centrifuge tube containing the WBCs. After thorough mixing, Proteinase K solution (40 µL) was mixed again.

The centrifuge tube was positioned in a 70 °C water bath for complete lysis of red blood cells. After observing the solution in the test tube becoming clear, a short centrifugation was performed to remove water droplets from the centrifuge tube walls. Then, anhydrous ethanol (400 µL) was added, followed by shaking on an oscillator for 15 min. After a brief centrifugation, the mixture (including precipitate) from the centrifuge tube was added to the QIAamp Mini spin column, which was then placed in a collection tube, which was covered and centrifugated at 8,000 rpm for 1 min and then discarded. Buffer AW2 (500 µL) was mixed with spin column, a 3-min continued centrifugation (14,000 rpm) was followed. The spin column was placed into a new 1.5 mL collection tube, centrifuged, and the collection tube and its contents were discarded. The spin column was then transferred to another new collection tube. Buffer EA (200 µL) was added, and after a 60-second incubation, a 1-min centrifugation was performed (8,000 rpm). The collection tube was stored at -20 °C.

The polymorphic genes rs6218, rs35767, and rs574261 of IGF-1 were analyzed using the MassARRAY system. Primers were designed based on sequence information obtained from GenBank. Specifically, the forward and reverse primers for rs6218 were 5'-TTCRCCAAGATGGCACTTCTTTT-3' and 5'-TCTGCAGAAGACTGCRCTATAAAGTTT-3', respectively; those for rs35767 were 5'-TTGGGCACATAGTAGAGCTCAC-3' and 5'-CAAAAGCRCCAGAGCAGACAT-3', respectively; and those for rs574261 were 5'-GGTTTTACAGCTCGGCATAGTC-3' and 5'-TCTGCTGGGCATGAAGACAC-3', respectively. The 50µl PCR reaction mixture comprised DNA framework 50µl, 10×PCR buffer 5 µl, 50mM Mgcl2 1.6 µl, 2mM dNTPs 4µl.

PCR amplification was conducted using following conditions: An initial denaturation step at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, and extension at 72 °C for 30 sec, concluding with a final extension at 72 °C for 5 min. Upon completion of the amplification, the samples were submitted to the company for sequencing. The sequencing results were compared with the reference sequences of normal IGF-1 genetic polymorphisms for rs6218, rs35767, and rs574261. This comparison aimed to identify the wild-type alleles and the corresponding mutant alleles at specific sites associated with these genetic polymorphisms.

Detection of related indicators in serum

The concentrations of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) in serum were determined using the Thermo Scientific™ Indiko™ fully automated biochemical analyzer. The CL of serum IGF-1 was measured using an enzyme-linked immunosorbent assay (ELISA), with three replicate measurements.

Statistical analysis

Data were processed using SPSS 26.0. Metric data were presented as means ± standard deviation, and inter-group comparisons were conducted using the independent samples t-test. Count data were expressed as percentages (%) and analyzed using the Chi-square (x^2) test. Meanwhile, Chi-square test was also employed to assess whether the IGF-1 SNPs conformed to the Hardy-Weinberg equilibrium (HWE). In the non-conditional logistic regression model, with CRC as the dependent variable, age, gender, family history of tumors, TG, TC, LDL, BMI, and smoking status were undertaken as covariates to analyze the correlation between the three IGF-1 SNPs and CRC. The relationship between genetic polymorphism of IGF-1 and its CL was analyzed using a linear regression model. Besides, an MR analysis model employed the inverse-variance weighted method (IVW) to estimate the relationship between SNPs and CRC. In addition, the MR Egger regression was utilized to assess the causal effect on CL of IGF-1 on CRC.

RESULTS

Table I shows general characteristics of the patients who participated in the study. The data suggested no great difference between subjects in the both groups in terms of gender, age, family history of tumors, BMI, or smoking status (P>0.05). On the other hand, the CL of IGF-1 in the CCG was greatly higher based on that in the CTG [(125.64±21.24) vs (109.26±19.35)], demonstrating a statistical significance with P<0.05.

Table I. General characteristics of subjects.

Index	CTG (n=320)	CCG (n=283)	P value
Male (n, %)	150(46.87)	141(49.82)	0.225
Female (n, %)	161(53.13)	142(50.18)	
Age (years)	62.64±6.27	61.25±6.51	0.116
Tumor history (n, %)	62(19.38)	57(20.14)	0.314
BMI>25	206(64.38)	184(65.02)	0.272
BMI≤25	114(35.62)	99(34.98)	
Smokers (n, %)	150(46.88)	127(44.88)	0.197
Non-smokers (n, %)	161(53.12)	156(55.12)	

Table II. Counts of IGF-1 in subjects in various groups.

Indicator	CTG (n=320)	CCG (n=283)	OR	P-value
SNPs rs6218				
UU	151	191	1.30	0.004
UC	100	100		
CC	32	29		
U	352	432		
C	114	108		
SNPs rs35767				
CC	143	136	1.16	0.006
CT	114	155		
TT	26	29		
C	343	350		
T	109	135		
SNPs rs574261				
TT	131	166	1.26	0.008
CT	120	122		
CC	32	31		
T	322	393		
C	124	123		

Table II illustrated the counts of three SNPs in different groups. As they illustrated, the IGF-1 SNPs in the populations in the CTG conformed to the HWE (P<0.05). A non-conditional logistic regression model was conducted for analysis, and the results revealed a visible correlation between rs6218 (OR=1.30, P=0.004), rs35767 (OR=1.16, P=0.006), and rs574261 (OR=1.26, P=0.008) with the CRC incidence.

The CTG was selected for analyzing the correlation between genetic polymorphism of IGF-1 and its CL, as demonstrated in Table III. It indicated that for IGF-1 rs6218, each additional risk allele (ARA) C for CRC was associated with an increase of 9.92 ng/mL in IGF-1 concentration (P=0.014). Similarly, for IGF-1 rs35767, each ARA T for CRC was associated with an increase of 8.14 ng/mL in IGF-1 concentration (P=0.025). However, for IGF-1 rs574261, where each ARA C for CRC was linked with a 6.2 ng/mL increase in IGF-1 concentration (P=0.126), observing no great difference. Upon comparison with data from the 1000 genome project database, it was observed that the genotype distribution frequencies of rs6218, rs35767, and rs574261 in the Chinese Han population conform to HWE without statistical significance (P>0.05).

Table III. Correlation between IGF-1 genetic polymorphism and IGF-1 circulating level.

Indicator	Genotype distribution (n)	IGF-1 (ng/mL)	β	x^2	P_{HWE}	P
SNPs rs6218						
UU	192	95±17.31	9.92	2.571	0.375	0.014
UC	100	112±22.48				
CC	29	132±25.13				
SNPs rs35767						
CC	165	91±11.42	8.14	1.628	0.273	0.025
CT	122	108±19.44				
TT	31	127±19.22				
SNPs rs574261						
TT	136	92±14.11	6.20	1.835	0.396	0.126
CT	156	116±22.17				
CC	30	137±26.55				

The MR analysis was conducted for rs6218 and rs35767 for analyzing the correlation between CL of IGF-1 related SNPs and the occurrence of CRC. In the case of mutation, each ARA for IGF-1 led to an increase in CRC incidence risk by 1.43% (P=0.039) and 1.28% (P=0.046) respectively, demonstrating obvious differences and significances. In the MR analysis of rs574261, when mutation occurred, each ARA for IGF-1 was linked with a 0.79% increase in CRC incidence risk (P=0.072) but exhibited no statistically great significance (Table IV).

Table IV. MR analysis of IGF-1 circulating level and CRC.

SNPs	Allele	β	Allele with 1 mutation	Allele with 2 mutations	OR (95% CI) value	P
rs6218	U/C	0.0143	1.126	1.296	1.014 (1.005-1.015)	0.039
rs35767	C/T	0.0128	1.104	1.163	1.013 (1.002-1.016)	0.046
rs574261	T/C	0.0079	1.045	1.094	1.008 (0.997-1.013)	0.072

DISCUSSION

This work focused on investigating patients diagnosed with CRC at our institution and individuals undergoing concurrent health check-ups. The MR analysis was employed to explore the correlation between the CL of serum IGF-1 and its genetic polymorphism with the incidence of CRC. Due to the challenge of early detection of CRC by patients, this work primarily was to analyze the potential variations of IGF-1 as a serum biomarker in the occurrence and progression of CRC. The results of this study revealed a remarkable link between IGF SNPs and an increased likelihood of CRC occurrence, as well as a notable correlation with the elevation of CL of serum IGF-1.

Animal and clinical studies have both confirmed an increased risk of CRC incidence associated with overexpression of IGF-1. Research suggests that early screening for CRC among individuals with elevated serum IGF-1 levels during health check-ups could potentially prevent progressions of CRC. IGF-1, a single-chain polypeptide synthesized by the liver under the stimulation of growth hormone, primarily facilitates bone development, growth, and maintenance of bone mass (Bademler *et al*., 2019). IGF-1 binds to its receptor, resulting in phosphorylation of tyrosine residues and subsequent docking with insulin receptor substrates. This activation triggers intracellular kinases, including MAPK and PI3K, initiating a cascade of amplification reactions within the cell. The activated substrates influenced by IGF-1 receptor and insulin receptor substrates impact downstream signaling, and studies suggest that elevated expression of insulin receptor substrate-1 appears to inhibit cell differentiation in CRC, while elevated insulin receptor substrate-2 promotes the transformation of intestinal epithelium to adenocarcinoma. Furthermore, increased insulin receptor phosphorylation levels promote the transition of normal colorectal epithelium to CRC, indicating the involvement of insulin receptors in carcinogenesis (Gligorijević *et al*., 2022). In exploring the role of human growth hormone (rhGH) in inflammatory bowel disease (IBD), elevated levels of IGF-1 were observed, indicating an association between the growth hormone/IGF-1 axis markers and cytokines such as interleukins IL-2 and IL-4 in pediatric IBD. However, rhGH treatment did not correlate with significant changes in a range of pro-inflammatory and anti-inflammatory cytokine levels (Wong *et al*., 2015). Metabolic syndrome (MetS) and its serum components, including elevated blood sugar levels, elevate the risk of developing CRC in patients. *In vitro* models of CRC have indicated that IGF-1 regulates glucose metabolism and influences the Warburg effect. The specific impact of IGF-1 on glucose metabolism within CRC seems to be influenced by its localized action, leading to the inhibition of CRC cell proliferation and the promotion of apoptosis through the inactivation of the TGF-β/PI3K/Akt/mTOR SPW (Kasprzak, 2021; Li *et al*., 2018). A semi-quantitative immunohistochemical study comparing the expression of IGF-1Ec in CRC and polyps with normal colon tissue found significantly elevated expression of IGF-1Ec in CRC and colorectal polyps compared to normal colon tissue. IGF-1Ec expression was even higher in colon adenomas with high-grade dysplasia, indicating its potential role in adenoma progression (Alagaratnam *et al*., 2020). Additionally, in cellular-level research by Hosseini *et al*. (2019) curcumin was found to downregulate MYC, insulin, and IGF-1 receptors. MYC, insulin, and IGF-1 receptor were sharply elevated in 5-FU chemotherapy-resistant cells. Downregulating the expression of epidermal growth factor receptor (EGFR) and IGF-1R could induce apoptosis in cancer cells (Codony-Servat *et al*., 2017). A large-scale study investigating the relationship between IGF-1 and CRC included 764 cases of advanced left-sided colon adenomas and 775 matched controls.

The results demonstrated a remarkably positive correlation between CL of IGF1 and risk of advanced colon adenomas. Similarly, the findings of this work indicated that elevated IGF-1 is a risk factor for CRC occurrence, aligning with the findings of Gao *et al*. (2021). The MR analysis suggests that elevated IGF-1 expression may contribute to CRC pathogenesis, with a positive correlation between CL of IGF-1 and CRC occurrence. These results align with Murphy *et al*. (2020) who found a strong association between the standard deviation increase in serum IGF-1 levels and higher CRC risk. Jiang *et al*. (2015) discovered that the SNP rs6218 of IGF-1 exerts a regulatory role in IGF-1 expression by inhibiting miR-603, promoting tumor regulation. In vitro and animal experiments have shown that IGF-1 is involved in CRC development and growth. A study revealed that SNP rs35767 related to IGF-35767 levels may be associated with CRC susceptibility, with individuals carrying the CT genotype potentially having a higher risk of CRC and often exhibiting poorly differentiated tissue (Li *et al*., 2018). Chen *et al*. (2013) found that non-coding microsatellite polymorphisms can serve as functional units that interact with SNPs in human genome transcription regulation. In an in vitro system, they compared the transcriptional activity of IGF-1 promoter evolutionarily conserved regions containing haplotypes (rs35767: T>C, CA repeat microsatellite; rs5742612: T>C; and rs2288377: T>A). The longest microsatellite, containing 21 repeats, exhibited the highest transcriptional activity in C-T-T haplotype, indicating its potential functional impact. In

the context of IGF-1 genetic polymorphism and CRC risk, this work found a tight correlation between the three IGF-1 SNPs rs6218 (OR=1.30, *P*=0.004), rs35767 (OR=1.16, *P*=0.006), and rs574261 (OR=1.26, *P*=0.008) and the risk of CRC. In terms of the correlation between IGF-1 genetic polymorphism and CL of IGF-1, it was observed that each ARA of IGF-1 rs6218 led to an obvious increase of IGF-1 concentration, as did each ARA of IGF-1 rs35767. In the context of correlations between CL of IGF-1 and CRC, the MR analysis demonstrated that each ARA of IGF-1 increased the risk of CRC occurrence by 1.43% and 1.28%, respectively, showing statistically obvious difference.

CONCLUSION

Results of this work demonstrated a tight association between elevated serum IGF-1 levels and the occurrence and progression of CRC. It highlighted the important role of IGF-1 genetic polymorphism, revealing a clear link between the SNPs and CRC incidence. Furthermore, the increase in alleles for IGF-1 SNPs rs6218 (U/C) and rs35767 (C/T) mutations was associated with an elevated risk of CRC development. The genetic polymorphism mutations in the IGF-1 gene may potentially serve as underlying indicators for CRC. These findings contributed to the identification of high-risk populations and provided crucial evidence for personalized prevention and treatment strategies. However, following research is warranted to elucidate the specific mechanisms of IGF-1 in CRC and its potential clinical applications.

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IRB approval

This study was approved by the Advanced Studies Research Board of Lishui People's Hospital, Lishui 323000, Zhejiang Province, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of Lishui People's Hospital, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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