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Exploration of the Causal Associations Between Circulating Inflammatory Factors, Metabolites, And Cervical Cancer: A Bidirectional Mendelian Randomization Study And Mediation Analysis

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ABSTRACT

Although numerous studies have demonstrated the critical role that inflammatory factors play in the onset and progression of different malignancies, their causal relationship with cervical cancer and the putative metabolite mediators is still unknown. Our study design incorporates the direct correlation and mediating role of metabolites in the relationship between inflammatory factors and cervical cancer. The data for this article comes from multiple queues and consortia, including the FinnGen database. We conducted comprehensive pairwise genetic analysis and used a mediated Mendelian randomization (MR) design to assess the causal effect of inflammatory factors on cervical cancer. The causality in mediation analysis is optimized in this work. To investigate the causative relationship between inflammatory factors and cervical cancer, as well as the mediating function of metabolites through inverse variance weighted (IVW), mainly a two-step, bidirectional Mendelian randomization research was utilized. As supplementary analysis techniques, other techniques, including MR-Egger, Weighted median (WM), Simple mod, and Weighted mode were also used. One inflammatory factor (C-C motif chemokine 28) was found to have a protective effect on CC by MR analysis. These inflammatory factors may also have an impact on CC via changing the metabolite (Alpha−hydroxyisovalerate). When it comes to cervical cancer, the mediated impact of alpha-hydroxyisovalerate is 0.0417(-0.0989, 0.182) and the causal effect of C-C motif chemokine 28 is the total effect IVW: OR = 0.606, 95% CI [0.397–0.926], p = 0.020. -8.34% is the mediated fraction (19.8%, -36.4%). This finding points to an alpha-hydroxyisovalerate-mediated causal link between C-C motif chemokine 28 and cervical cancer. Our results demonstrate the intricate connection between metabolites, inflammatory factors, and cervical cancer. The associations and mediating effects that have been identified provide valuable insights into potential therapeutic pathways targeting inflammatory factors for the management of cervical cancer.

KEYWORDS

Inflammatory factors; Cervical cancer; Mendelian randomization; Mediation analyses; Metabolite

1. INTRODUCTION

Cervical cancer is the fourth most common cause of cancer-related deaths in women and ranks 14th overall in terms of cancer types. Its prognosis will be poor since it will miss the best chance for surgery, radiation, and other combination therapies because of the failure to receive an early diagnosis.

The survival rate for cervical cancer has increased over time due to the introduction of vaccines and advancements in treatment techniques [1]. In spite of this, over 600,000 people die from cervical cancer each year [2]. There is a disparity in the morality of this illness. The incidence of cervical cancer is seven to ten times higher in developing nations like sub-Saharan Africa, Melanesia, and South America than in industrialized nations like North America and Australia [3, 4]. The focus of cervical cancer interventions is mostly on primary and secondary preventive strategies. Using preventative screening and primary prevention is the best strategy to reduce the injuries and mortality that cervical cancer causes [5]. As a result, the most economical, effective, and safest way to treat cervical cancer is through early-stage, more accurate screening. It is hypothesized that by providing nutritional signals, a low-inflammatory state can increase the rate of mutation and boost the proliferation of mutant cells, hence boosting the growth of tumors. Studies have demonstrated that inflammation becomes a characteristic of cancer [6-8].

An essential function for inflammatory reactions is played in the initiation and spread of cancer. Certain kinds of cancer share a molecular base, according to certain research. Higher levels of C-reactive protein (CRP) in the bloodstream, for instance, have been connected to an increased risk of malignancies of the breast, lung, prostate, and numerous other organs. Interleukin-1 (IL-1a) and IL-8 have been linked to an increased risk of ovarian cancer [9]. The intricate interplay of cytokines, chemokines, and active lipids orchestrates the process of inflammation across multiple cellular regions, characterized by the activation and persistent escalation of inflammatory channels in cervical cancer [10]. If the connection between inflammation and cancer originates from the capacity to precisely establish causation, this may function as a means of reducing the likelihood of cancer. Cervical cancer development is also significantly influenced by inflammatory factors. The viral oncogene causes prostaglandin E2 (PGE2), the COX enzyme, to be synthesized when the virus fuses with cervical epithelial cells. PGE2 regulates the activity of tumor cells through the cAMP signaling system [11]. However, there is insufficient information to evaluate and validate the actual physiological significance of the most prevalent inflammatory variables in cervical cancer yet.

Despite the fact that tumor-specific metabolic alterations have only been observed for almost a century, there has been a general increase in interest in the metabolic study of cancer. It is now very difficult to examine how the metabolism is evolving in each form of tumor because they all have different metabolic processes. Variations in the local concentrations of metabolites inside the tumor microenvironment (TME) could potentially exert a substantial impact on the tumor's growth [12, 13]. A few key phases of the cell's metabolism are altered by tumor-related metabolic alterations, and the differentiation of cancer cells and the makeup of the tumor microenvironment both have a significant impact on the cell's destiny due to the preferential distribution of nutrients along metabolic pathways [14]. Research has revealed that some metabolites, like chain fatty acids (SCFAs), are also crucial in the etiology of a number of inflammatory illnesses, including colon cancer and inflammatory bowel disease [15]. Metabolites function as minuscule molecules in intermediate or final products during metabolic processes. These substances exhibit both biological and pharmacological effects, potentially influencing disease risk, and are a key focus for therapeutic strategies [16]. Numerous metabolites can impact tumor progression: glucose metabolism plays a role in cancer immune surveillance; amino acid metabolism is linked to tumor inflammation and T cell diversification; and fatty acids and cholesterol play a critical role in intercellular communication within the tumor microenvironment. Metabolite signal transduction is an essential process that affects mesenchymal transition in epithelial cells, cellular proliferation, malignant alterations, cell differentiation inhibition, and cancer dryness regulation. Additionally, within the tumor's microenvironment, metabolite signaling between cells plays a critical role in regulating inflammatory responses and immune surveillance [17–19]. Comprehensive knowledge of the role metabolites play in the pathogenesis of disease can lead to intervention strategies that are successful in treating the condition. Numerous disease-related biomarkers have been analyzed and compared using metabolomics techniques, and risk factors that may increase the chance of cancer or other health problems have also been predicted [20-22].

Even though modern technologies are able to treat these patients with greater speed, in some cases, the majority of cancer treatments, including radiation, chemotherapy, immunotherapy, and surgery, have a poor prognosis and extremely harmful side effects that include pain, nausea and vomiting, fatigue, depression, hair loss, and weakened immune systems [23]. Thus, the need for novel, safer, and more effective treatments is urgent. In order to choose the most relevant therapeutic indicators and thus aid in the treatment of cervical cancer, this paper uses Mendelian randomization [24–26]. We are able to evaluate the role that metabolites play in the outcomes of disease by means of human genetics. Mendelian randomization finds favorable conditions thanks to this characteristic [27].

Mendelian randomization (MR) is gaining popularity because of its causal-based reasoning methodology, which makes it possible to analyze and evaluate possible risks associated with exposure to outcomes [28]. Research on the possible effects of exposure, such as inflammatory agents and metabolites, on the final course of the disease is conducted using genetic variation. This randomization strategy usually reduces the skewness and partiality of confounding factors due to the stochastic allocation of alleles throughout pregnancy [29].

Seeing that it is still unclear how inflammatory factors influence the progression of cervical cancer through metabolite regulation. We employed mediated and bidirectional Mendelian randomization as a research design to thoroughly examine the potential causative association between inflammatory variables and cervical cancer via particular metabolic pathways. This research involved gathering information on single nucleotide polymorphisms (SNPs), serving as instrumental variables (IV) to reveal research methodologies. Next, we performed a comprehensive circulating and mediated Mendelian randomization analysis to estimate the causal role of inflammatory factors and metabolites in the pathogenesis of cervical cancer. Subsequently, we examined the impact of inflammatory factors on the risk assessment of cervical cancer, mediated through metabolite signaling, to determine if these cytokines can affect the advancement of cervical cancer by modifying metabolites.

2. METHOD

2.1. Study Design

To explore the causal link between systemic inflammatory factors and cervical cancer, we conducted a bidirectional Mendelian study. Furthermore, mediation studies were performed to investigate the potential mediating role of metabolites in the relationship between cervical cancer risk and inflammatory variables. Two-step Mendelian randomization might be the most suitable method if the main objective of causality is to assess the various roles of several mediating variables and their potential impacts on a single mediating variable [30]. Using a two-sample MR investigation, the first phase involved evaluating the causative effects of metabolites and inflammatory variables on cervical cancer and screening for genetic characteristics highly related to the risk of cervical cancer. The subsequent phase involves assessing the impact of chosen inflammatory elements on the traits of the examined metabolites and determining the intermediary influences and impacts of each factor on cervical cancer. Every piece of data utilized in this research was derived from earlier studies, and informed consent was secured during the initial survey. The three fundamental presumptions of MR analysis were strictly adhered to when operating the instrumental variables that we chose: (a) The hypothesis of association states that there is a high correlation between exposure variables and SNPs. (b) Independence hypothesis: confounding factors have no effect on SNPs (c) The exclusion hypothesis states that exposure factors are the only way SNPs can affect results [31]. (Figure 1)

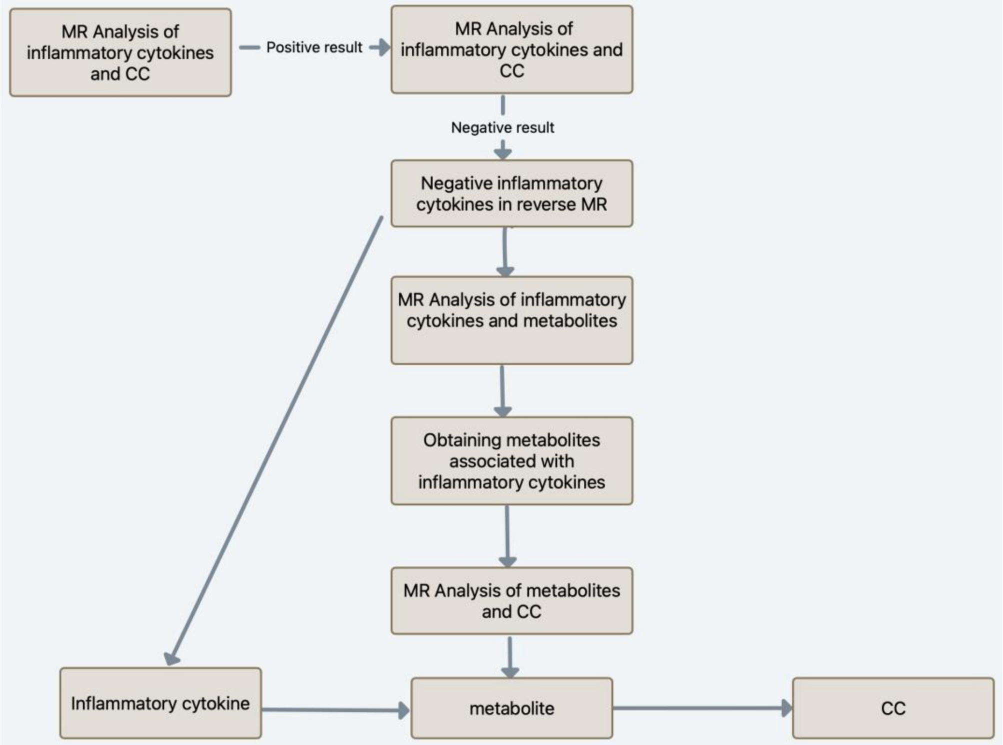


Figure 1. Schematic representation of MR research

2.2. Data Source

We employed patients with cervical malignant lesions from the FinnGen study, comprising 388 cases and 182,927 controls, to conduct research. The 10th edition of FinnGen data contained 412,181 samples totaling 230,310 women and 181,871 men of GWAS data (Genome-Wide Association Studies). It refers to large-scale, multicenter, and consistently validated research on the relationship between genes and disorders at the whole-genome level. The resource encompasses forward-looking epidemiological groups, cohorts based on diseases, and samples from hospital biobanks [32]. The objective of the project is to employ Genome-Wide Association Studies genotyping to produce almost comprehensive genomic variant information for half a million participants in the biobank. GWAS describes multi-center studies using large samples and repeated verification at the entire genome level to investigate the relationship between genes and diseases. Using large-scale population DNA samples, high-density genetic markers (such as SNP or CNV, etc.) are typed throughout the entire genome in an effort to find genetic components linked to complex disorders. Completely identify the hereditary genes involved in the genesis, progression, and management of illnesses. In addition, this study used 1400 metabolites of summary statistics numbers for GCST90027001-GCST90028000 / GCST90027857/GCST90027446-GCST90027857, and the data are available at http://ftp.ebi.ac.uk/pub/databases/gwas summary statistics/gwas. In addition, another part of the data comes from https://www.phpc.cam.ac.uk/ceu/proteins and the EBI GWAS Catalog (accession numbers GCST90274758 to GCST90274848). Statistics: 91 inflammatory factors GWAS data from https://www.phpc.cam.ac.uk/ceu/proteins and the EBI GWAS Catalog (accession numbers GCST90274758 to GCST90274848), and all participants are from Europe. The individual-level genetic and proteomic data of the INTERVAL cohort are stored in the European Genome Phenoome Archive with entry number EGAS00001002555. The gene expression data is stored in GEO with the entry number GSE16879.

2.3. Genetic Instrumental Variables (Ivs) Selection

Finding SNPs that are substantially linked to inflammatory agents or metabolites and cervical cancer is the next stage. A stricter criterion was chosen to reduce the range since there are too many inflammatory variables and metabolite-related SNP. A genome-wide significance criterion of P<1e-05 was established. And it could be better to utilize a lower threshold in order to prevent over-merging related sites, since the data set in this work contains a comparatively high density of genomic regions and linkage disequilibrium (LD). We decided to adjust LD parameters for SNPs significantly associated with exposure to the conditions r 2<0.001 and kb > 1000. We set a parameter threshold of P<1e-05 during the reverse Mendelian randomization method to filter out inflammatory components that produced negative findings. To ensure the independence of the selected IV, we used strict r2<0.1 and kb>500 criteria to rule out the possibility of strong linkage imbalances in the SNPs. These strict parameters can properly balance the detection power and false positive rate. Utilizing MR-PRESSO enables us to identify and rectify horizontal pleiotropy through the removal of anomalies, and palindromic SNPs with intermediate allele frequencies were eliminated. Additionally, F-statistics were employed to assess the effect of genetic instruments on all SNPs. SNPs with F-values less than 10 were regarded as weak instrumental factors and excluded from a more in-depth MR analysis [33].

2.4. Statistical Analysis

Mendelian randomization analysis utilizing the "ieugwasr" package, "VariantAnnotation" package, and "TwoSampleMR" package in R (version 4.3.0). We used inverse variance weighting (IVW) as the primary analysis to determine the causal relationship between cervical cancer and inflammatory factors. At the same time, several other methods, such as MR-Egger, Weighted median (WM), Simple mod, and Weighted mode are adopted as auxiliary analysis methods [34]. The oldest and most widely applied technique is IVW. Its feature is that the weight to fit is determined by taking the reciprocal of the outcome variance instead of accounting for the presence of an intercept term in the regression. In comparison to the IVW technique, estimates obtained using the MR-Egger method may be more biased and have a greater type 1 error rate when the pleiotropic effects of numerous genetic variants operate through the same confounders [35]. And the weighted average of each genotype is determined by the weighted mode approach, which allows for a thorough evaluation of the impact of various genotypes on phenotypes. Moreover, a comparison of the frequency or proportion of genotypes or phenotypes between the control and experimental groups is typically presented in the Simple mod result. It is able to display the genes that have been observed [36]. Another popular supplemental analytic technique in MR studies is the weighted media approach, which is determined by taking the median estimate of variation specificity [37]. P<0.05 denotes a substantial causal connection between the outcomes and exposure. OR and 95% CI were used to show the impact of inflammatory agents and metabolites on the risk of cervical cancer. Positive beta (effect size) indicates that exposure increases lead to an increase in the outcome variable. IVW-derived estimates are preferred when there is no heterogeneity or pleiotropy. As an MR technique, the IVW approach evaluates the combined effect of several loci in multiple SNP analyses. Verifying each SNP's validity as an instrumental variable and its complete independence from the others is the main goal of IVW. In cases of sole heterogeneity without pleiotropy, the Weighted Median approach is favored, and the WM technique provides consistent and dependable calculations, provided that a minimum of 50% of the data originates from legitimate instrumental variables. MR-Egger avoids pushing the regression line through the origin, thereby permitting directional genetic pleiotropy in the instrumental variables involved [38]. The term "simple mod" typically denotes fundamental statistical frameworks that avoid intricate modifications or numerous variables, yet are employed in initial analyses to evaluate variables that have a direct correlation with one another [39].

2.5. Sensitivity Analysis

A sensitivity analysis was then performed to help us evaluate the robustness of our findings. Its effects is to assess the conclusions' dependability and robustness, check the results for bias (pleiotropy, heterogeneity of the data, etc.), and determine if an instrumental variable has a statistically significant impact on the outcome variable. Additionally, the "MR PRESSO" package was used to implement the MR pleiotropy residual sum and outlier approach (MR-PRESSO) for the purpose of identifying and rectifying horizontal pleiotropy. The variety in SNP estimations was assessed using the Cochran's Q test, which provides strong evidence for selecting suitable analytical methods. If the P-value is less than 0.05, which indicates that there is no heterogeneity, the fixed-effect IVW technique should be the first strategy to be considered; if not, other tests should be taken into consideration. The funnel plot needs to pay attention to confirm that the points on both sides of the IVW line are nearly symmetrical. If there are any obvious outliers, it may be necessary to consider removing them. We employed Cochran's Q value to identify heterogeneity between estimates; a $p < 0.05$ indicated the presence of heterogeneity. Additionally, we conducted a “leave-one-out” analysis to evaluate the substantial impact of each particular SNP on the outcomes. Estimates that are closer to the genuine value can be obtained by eliminating outliers and the relevant SNPs. Moreover, MR-Egger analysis also revealed horizontal pleiotropy [40].

2.6. Mediation Analysis

The mediating role of metabolites on the relationship between cervical cancer was evaluated through the use of mediating analysis. Inflammatory variables' overall impact on cervical cancer was observed. The "product of coefficients" approach was used to assess the indirect impact of putative inflammatory factor mediators on the risk of cervical cancer. Indirect effects, or mediating effects, denote the influence of metabolites, and the ratio of mediating effects is determined by dividing these effects by the overall effects. Factors related to inflammation can affect disease risk through changes in specific metabolite levels. The delta method was used to ascertain the standard errors linked to indirect impacts [41].

3. RESULT

3.1. Risk Analysis of Inflammatory Factors for Cervical Cancer

Although statistical significance was not reflected by other methods, the IVW method showed that increased Adenosine Deaminase was associated with a lower risk of cervical cancer (odds ratio (OR) = 0.7342) with a 95% Confidence Interval (CI) [0.5426−0.9936], P<0.05). IVW method detected in C−C motif chemokine 28 (OR = 0.6062, 95% CI [0.3970−0.9257], P<0.05). The IVW method was used to obtain TNF−related apoptosis−inducing ligand (OR = 0.7800, 95%CI [0.6086−0.9996], P <0.05) (Figure 2(A)-(H)), and Weighted median, Weighted mode analysis results showed that P<0.05. These results indicate the protective effects of Adenosine Deaminase, C-C motif chemokine 28, TNF-related apoptosis−inducing ligand, and other inflammatory factors on cervical cancer. Matrix metalloproteinase−10 was determined by the IVW method (OR = 1.4938, 95% CI [1.0303−2.1659], P<0.05) and was determined to have a negative causal effect on cervical cancer. (Figure 3; Table S1)

Forest maps showed a causal relationship between inflammatory factors significantly associated with cervical cancer; The analytical method was IVW, MR Egger, Weighted median, Simple mode, Weighted mode.

The application of Cochrane's Q-test, MR-Egger intercept test, and MR-PRESSO techniques revealed the absence of diversity or horizontal pleotropy in this MR analysis (Figure 2 (I)–(L)). Furthermore, employing the leave-one-out analytical approach revealed that none of the SNPs significantly deviated from the general influence of inflammatory elements on cervical cancer (Figure 2 (M)–(P)).

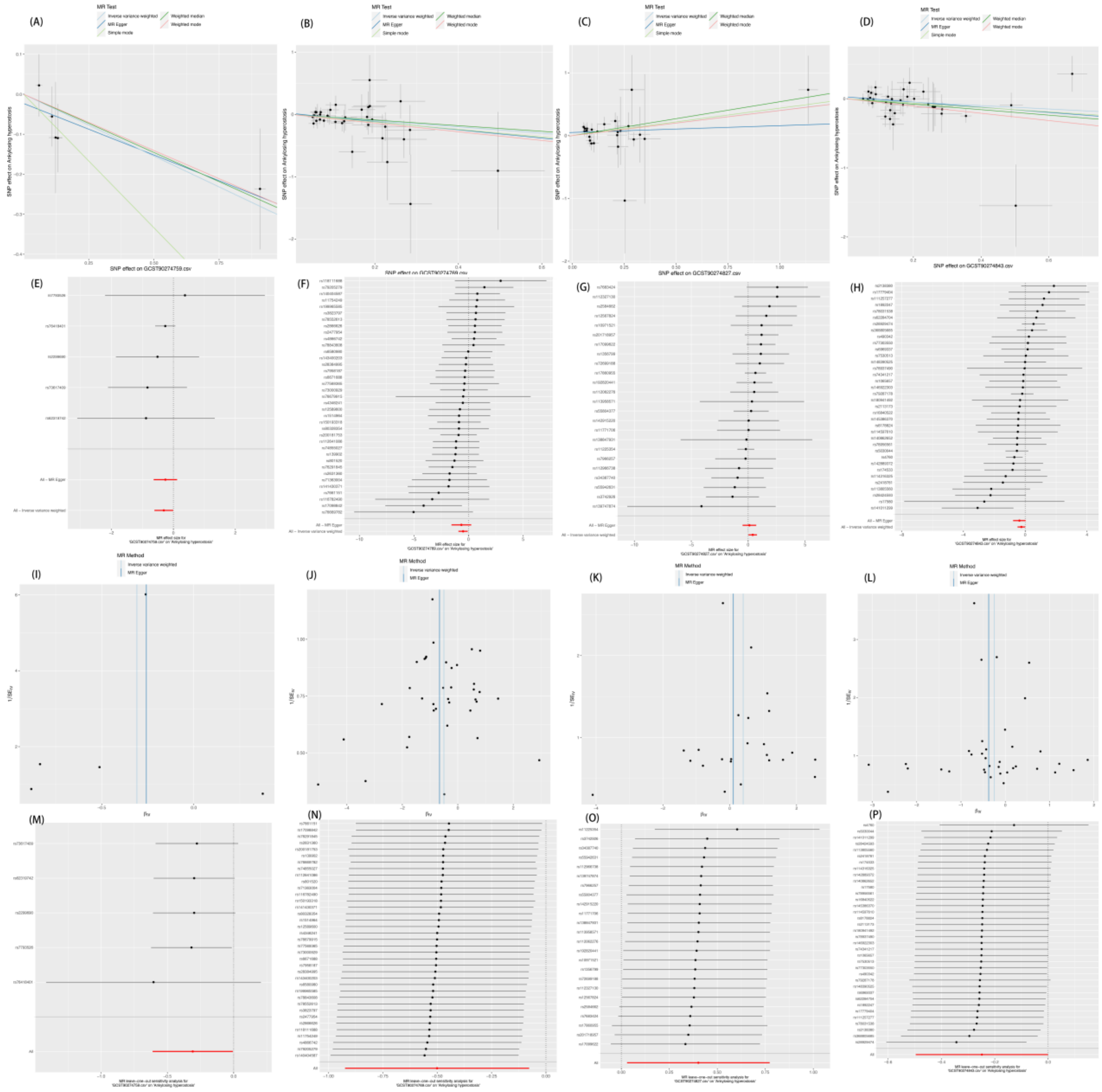


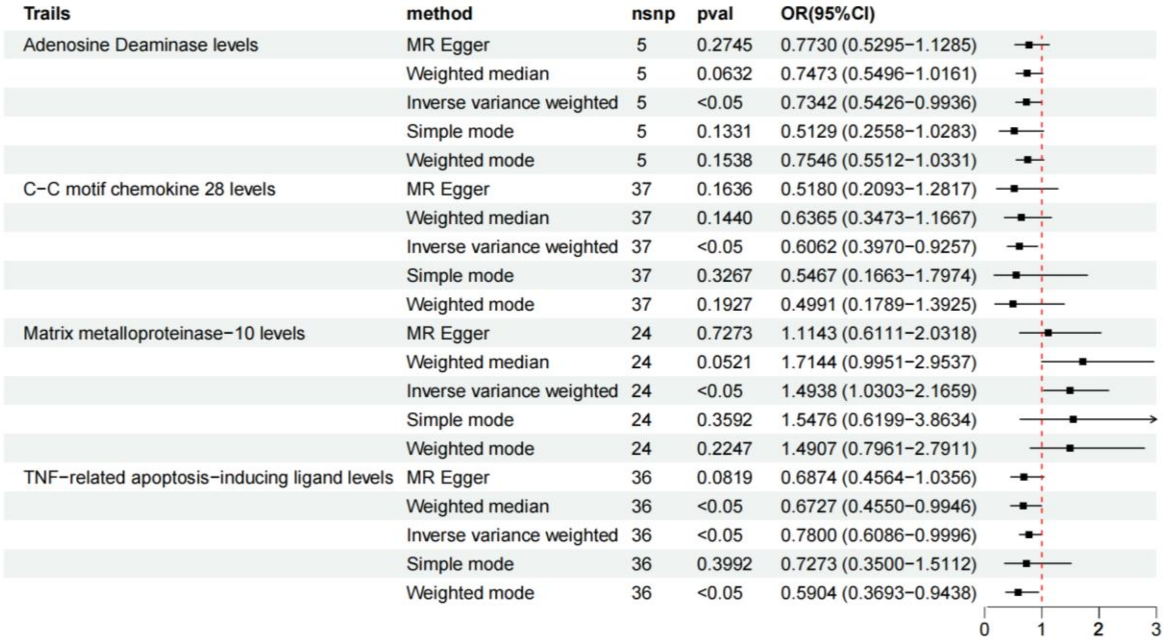
Figure 2. Mendelian randomization of inflammatory factors

(A)-(D): Scatter plots of inflammatory factors strongly associated with cervical cancer .

(E)-(H): Forest map inflammatory factors strongly associated with cervical cancer and cervical cancer.

(I)-(L): Funnel plot for sensitivity analysis of inflammatory factors strongly associated with cervical cancer.

(M)-(P): Four types of gut microbiota closely associated with cervical cancer were mapped by “leave-one-out” method.



Trails	method	nsnp	pval	OR(95%CI)
Adenosine Deaminase levels	MR Egger	5	0.2745	0.7730 (0.5295−1.1285)
	Weighted median	5	0.0632	0.7473 (0.5496−1.0161)
	Inverse variance weighted	5	<0.05	0.7342 (0.5426−0.9936)
	Simple mode	5	0.1331	0.5129 (0.2558−1.0283)
	Weighted mode	5	0.1538	0.7546 (0.5512−1.0331)
C−C motif chemokine 28 levels	MR Egger	37	0.1636	0.5180 (0.2093−1.2817)
	Weighted median	37	0.1440	0.6365 (0.3473−1.1667)
	Inverse variance weighted	37	<0.05	0.6062 (0.3970−0.9257)
	Simple mode	37	0.3267	0.5467 (0.1663−1.7974)
	Weighted mode	37	0.1927	0.4991 (0.1789−1.3925)
Matrix metalloproteinase−10 levels	MR Egger	24	0.7273	1.1143 (0.6111−2.0318)
	Weighted median	24	0.0521	1.7144 (0.9951−2.9537)
	Inverse variance weighted	24	<0.05	1.4938 (1.0303−2.1659)
	Simple mode	24	0.3592	1.5476 (0.6199−3.8634)
	Weighted mode	24	0.2247	1.4907 (0.7961−2.7911)
TNF−related apoptosis−inducing ligand levels	MR Egger	36	0.0819	0.6874 (0.4564−1.0356)
	Weighted median	36	<0.05	0.6727 (0.4550−0.9946)
	Inverse variance weighted	36	<0.05	0.7800 (0.6086−0.9996)
	Simple mode	36	0.3992	0.7273 (0.3500−1.5112)
	Weighted mode	36	<0.05	0.5904 (0.3693−0.9438)

Figure 3. Forest map of inflammatory factors

3.2. Inverse Mendelian Randomization Analysis

When inflammatory factors were taken as a result, cervical cancer did not have a clear causal link between Adenosine Deaminase (OR = 1.10, 95% CI [0.984–1.037], P = 0.456) and Adenosine Deaminase, as shown in the figure. The results showed C−C motif chemokine 28 (OR = 0.557, 95% CI [0.970–1.021], P = 0.700), indicating that cervical cancer is not clearly associated with it. Furthermore, Matrix metalloproteinase−10 was not found to be significant in the diagnosis of cervical cancer (OR = 1.008, 95% CI [0.982–1.035], P = 0.557). By analyzing the causal relationship between cervical cancer and TNF-related apoptosis-inducing ligands (OR = 1.007, 95% CI [0.974–1.041], P = 0.687), we can conclude that there is no clear causal link between the two. (Figure 4(A)-(H)) At the same time, no heterogeneity or level of pleiotropy was observed. (Figure 4(I)-(P))

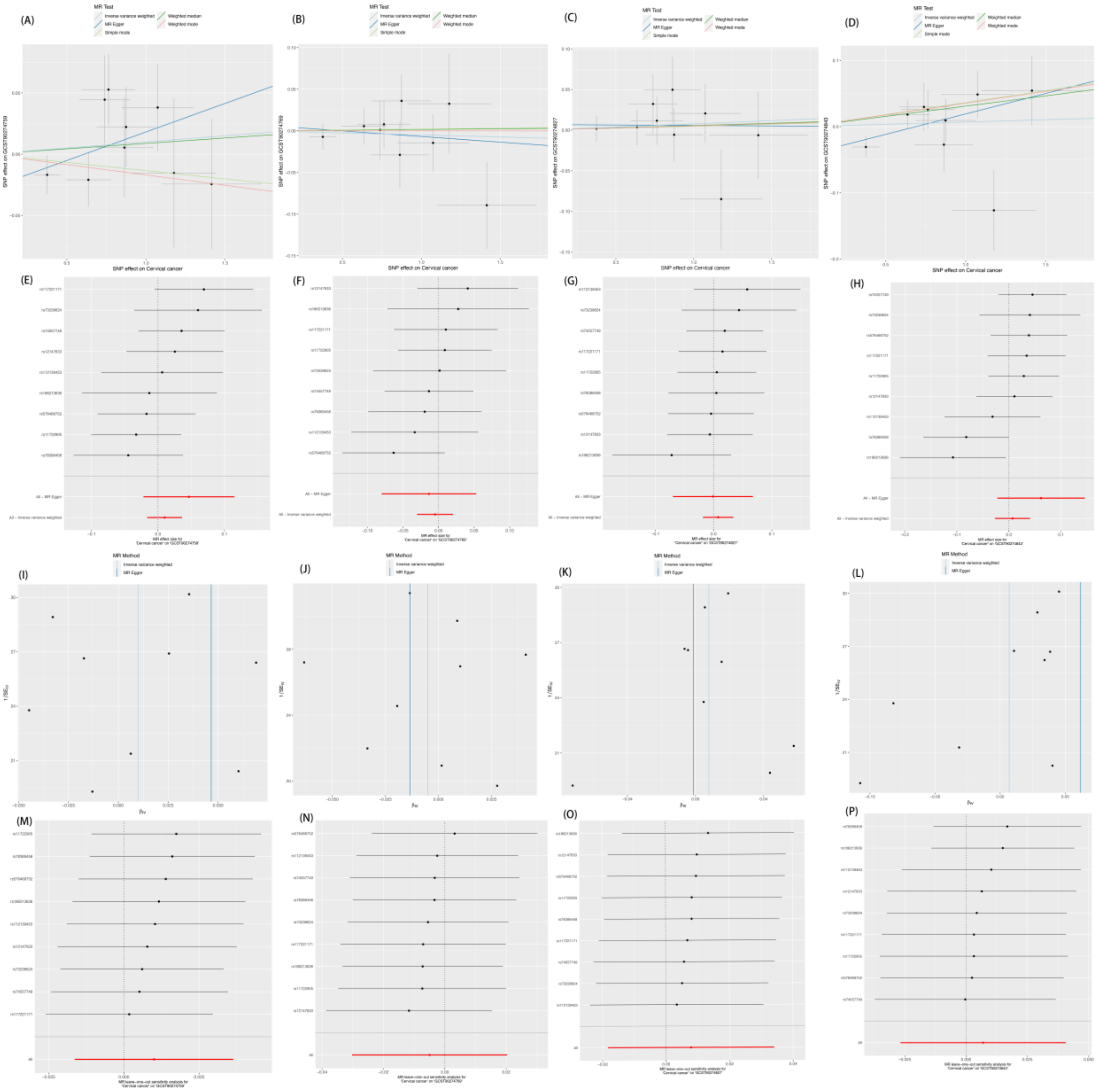


Figure 4. Inverse Mendelian randomization of inflammatory factors and cervical cancer

(A)-(D): Scatter plots of cervical cancer strongly associated with inflammatory factors .

(E)-(H): Forest map CC strongly associated with cervical cancer and inflammatory factors.

(I)-(L): Funnel plot for sensitivity analysis of cervical cancer strongly associated with inflammatory factors.

(M)-(P): Cervical cancer closely associated with inflammatory factors were mapped by “leave-one-out” method.

3.3. Risk analysis of Inflammatory Factors and Metabolites

The effect of 1400 metabolites on the risk of cervical cancer was determined using the IVW approach; supplemental verification was conducted using the MR-Egger, Weighted median (WM), the Simple mod, the Weighted mode, and other methods. The consistency of the outcomes was assessed using the OR values that these five methods produced. Ultimately, pleiotropic metabolites were eliminated in order to screen nine representative metabolites linked to cervical cancer. Seven of them, including 1-palmitoyl-GPC, pyridoxate, and 5-hydroxylysine, were positively correlated with the risk of cervical cancer. 3-(3-amino-3-carboxypropyl)uridine, pseudouridine, X-23665, and alpha-hydroxyisovalerate. The two metabolites 1-(1-enyl-Palmitoyl)-2-Linoleoyl-GPC and X-11847 are negatively linked to cervical cancer. Cochrane's Q-test yielded a P-value that was more than 0.05, indicating the absence of significant heterogeneity. According to leave-one analysis, the causal estimate remained unchanged when a specific SNP was eliminated. (Table S2)

Four inflammatory factor signatures that are essential for cervical cancer have previously been discovered. Next, we looked into how nine metabolites affected the characteristics of cervical cancer. One inflammatory component was shown to be highly correlated with metabolites (OR = 1.099, 95% CI [1.004-1.203], P = 0.0408), according to Mendelian analysis of the results of inflammatory factors closely associated with cervical cancer and metabolites significantly associated with cervical cancer.(Figure 5 (A, B)) Additionally, the weighted mode and weighted median approaches demonstrate a considerable causal effect. Weighted median: (OR=1.545, 95%CI [1.028-2.793], P = 0.015); Weighted mode: (OR=1.233, 95%CI [1.016-1.496], P = 0.041). According to the MR Egger method, there was no significant causal relationship between metabolites and inflammatory factors (OR = 1.181, 95% CI [0.988–1.412], P = 0.075). The results obtained by the simple mode method showed that there was no obvious causal relationship between inflammatory factors and metabolites (OR = 1.241, 95% CI [0.983–1.567], P = 0.077). There was no evidence of heterogeneity or horizontal pleiotropy, nor was there a single SNP that caused a causal change, so the results of this study are credible. (Figure 5 (C, D), Table S3)

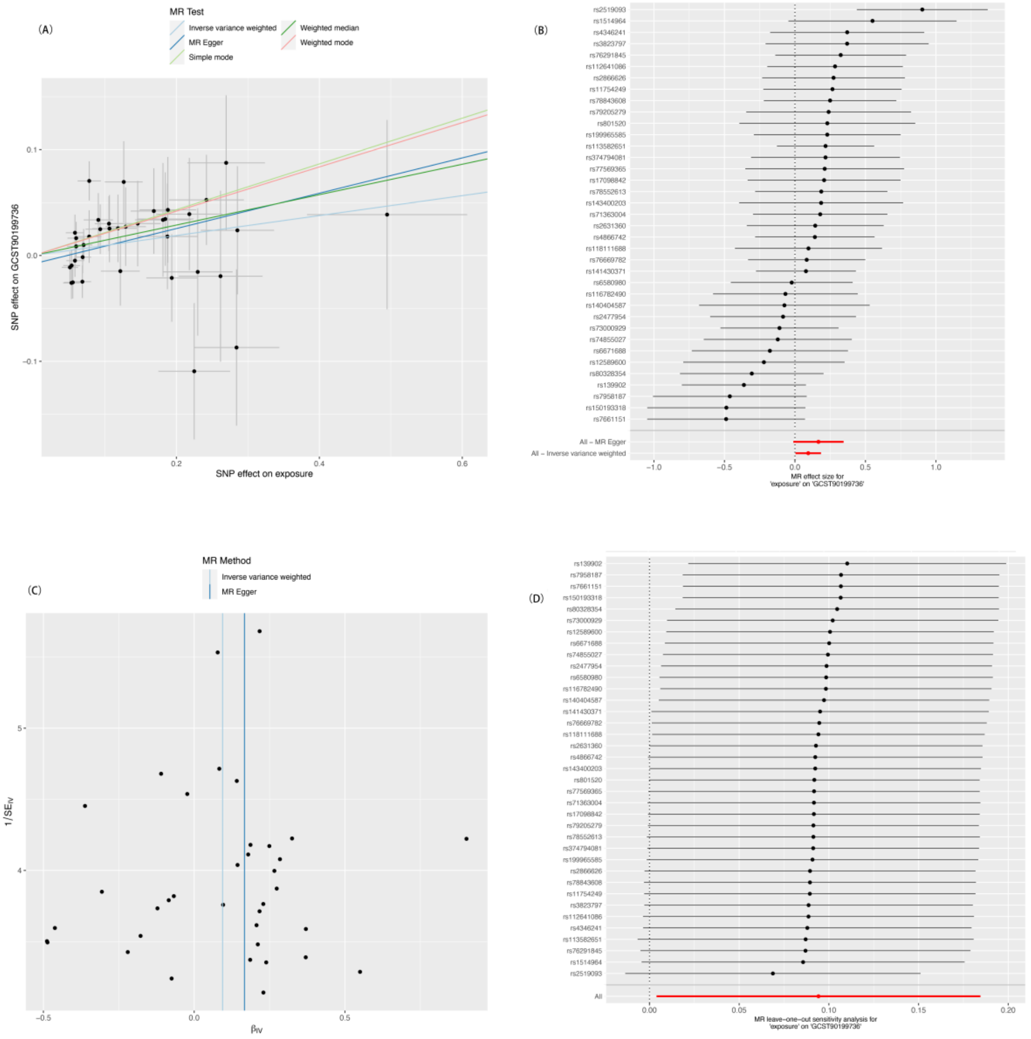


Figure 5. Mendelian randomization analysis of inflammatory factors and metabolites

(A): Scatter plots of C-C motif chemokine 28 strongly associated with Alpha−hydroxyisovalerate

(B): Forest map of C-C motif chemokine 28 strongly associated with Alpha−hydroxyisovalerate

(C): Funnel plot for sensitivity analysis of C-C motif chemokine 28 strongly associated with Alpha−hydroxyisovalerate.

(D): C-C motif chemokine 28 strongly associated with Alpha−hydroxyisovalerate were mapped by “leave-one-out” method.

CI: Confidence Interval; MR: Mendelian randomization; OR: odds ratio; SNP: Single nucleotide polymorphism

3.4. Risk Analysis of Cervical Cancer and Metabolites

After assessing 1,400 metabolites for cervical cancer risk, the IVW approach identified a clear association between 48 metabolites and the disease; 24 metabolites had a positive correlation and 24 had a negative one. Sensitivity analysis showed no pleiotropy or significant differences at any level. The total effect of inflammatory factors on cervical cancer was derived after using metabolites as mediators.

A description of the correlation analysis was given between the chosen inflammatory variables and cervical cancer. The C-C motif chemokine 28 and the incidence of cervical cancer were found to be significantly positively correlated using the IVW approach (OR = 0.606, 95% CI [0.397–0.926], p = 0.020). No significant association was found between C-C motif chemokine 28 and cervical cancer when tested by other complementary methods. When the MR Egger method is used (OR=0.518, 95%CI [0.209-1.282], p=0.164) and when the Weighted median method is used (OR=0.637, 95%CI [0.351-1.153], p=0.136), the simple mode method (OR=0.547, 95%CI [0.177-1.684], p=0.300) and the weighted mode method (OR=0.499, 95%CI [0.186-1.340], p=0.176) were adopted. (Figure 6 (A, B)) Based on the aforementioned findings, cervical cancer can be treated and prevented with the application of C-C motif chemokine 28 as a protective factor. These connections did not demonstrate heterogeneity, according to Cochran's Q test. Additionally, there was no evidence of any associated horizontal pleiotropy according to the MR-PRESSO test or the Egger intercept. Furthermore, we confirm the stability and validity of our findings by applying the "leave-one-out" strategy, which leads us to the conclusion that the absence of any single SNP factor has no effect on these causal correlations. Figure 6 (A, B)

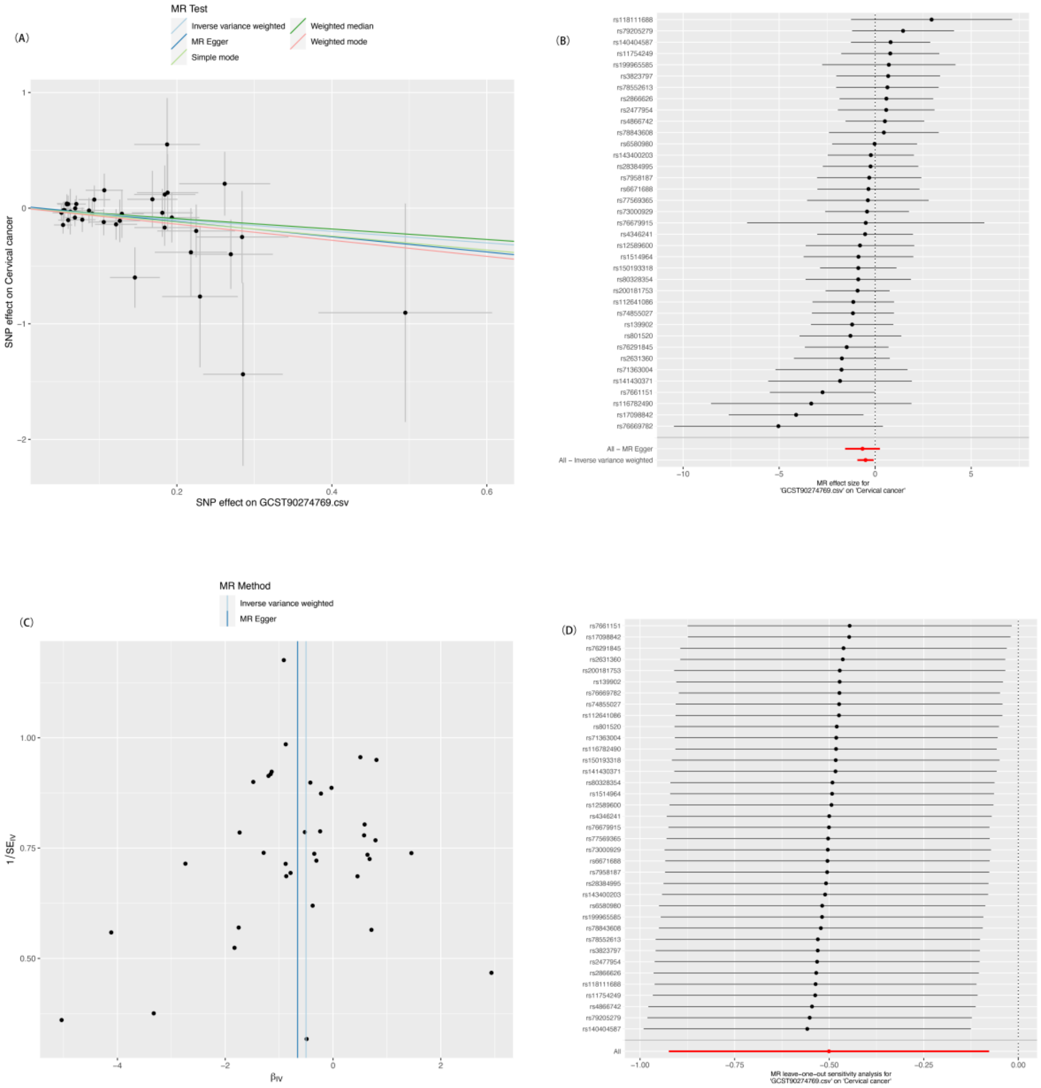


Figure 6. Total effect of Mendelian randomization of inflammatory factors and disease

(A): Scatter plots of C-C motif chemokine 28 strongly associated with CC.

(B): Forest map of C-C motif chemokine 28 strongly associated with CC.

(C): Funnel plot for sensitivity analysis of C-C motif chemokine 28 strongly associated with CC.

(D): C-C motif chemokine 28 strongly associated with CC were mapped by "leave-one-out" method.

CI: Confidence Interval; MR: Mendelian randomization; OR: odds ratio; SNP: Single nucleotide polymorphism.

3.5. Mediation Effect of Metabolites on CC

Previously, we analyzed the causal effect of the gene inflammatory factor C-C motif chemokine 28 on cervical cancer. Mediation analysis was performed to describe the mediated effect, and the results showed that the C-C motif chemokine 28 could be identified by the Alpha−hydroxyisovalerate metabolic pathway (OR = 1.099, 95% CI [1.004-1.203], P = 0.041) and affected the occurrence, development, and treatment of cervical cancer， and mediated effect is 0.0417(-0.0989, 0.182). Additionally, the mediated proportion is -8.34% (19.8%, -36.4%). In addition, we supplemented the IVW approach with the Weighted mode, Weighted median, MR Egger, and Simple mode method. This shows that C-C motif chemokine 28 may change the amount of Alpha−hydroxyisovalerate, which may affect the risk of cervical cancer. This particular metabolic pathway may also be a target for cervical cancer treatment in the future. Sensitivity analysis revealed no significant level of pleiotropy or difference.

4. DISCUSSION

In order to explore the causal link between inflammatory factors and cervical cancer, we used bidirectional Mendelian randomization research. Following stringent screening, we discovered that one inflammatory factor had negative effects on cervical cancer and three inflammatory factors had protective benefits. We carried out a two-step MR study to investigate further the impact of inflammatory variables on cervical cancer through metabolites or metabolic pathways. Initially, we identified nine metabolites that significantly increase the risk of cervical cancer. Second, we looked at how inflammatory variables affect metabolites causally. The findings demonstrated that the only inflammatory component with a substantial causal link to the metabolite Alpha-hydroxyisovalerate was C-C motif chemokine 28. Based on our findings, Alpha−hydroxyisovalerate may be an important regulator of the causal pathway from C-C motif chemokine 28 to cervical cancer risk. In addition, we used mediation analysis to calculate the proportion of indirect effects. The data showed that the mediated effect of Alpha−hydroxyisovalerate on C-C motif chemokine 28 was 0.0417 (-0.0989, 0.182). The median proportion was -8.34% (19.8%, -36.4%), suggesting that Alpha−hydroxyisovalerate was the key mediator between cervical cancer and C-C motif chemokine 28.

The inflammatory tumor microenvironment (TME) is formed by the carefully orchestrated interactions between cancer cells and the surrounding stromal and inflammatory cells [42].

Many studies have begun to understand the significance of inflammatory factors in the pathophysiological process of various diseases, especially the study of their relationship with cancer, which has received the attention of most scholars. Research indicates that chronic inflammation linked to tumors contributes to the immunosuppression of the tumor's immune microenvironment and its progression [43].

Chemokines are unique among inflammatory mediators in that they are generated as tiny proteins with molecular weights ranging from 8 to 10 kDa. A variety of chemokines are essential for controlling the immune system's reaction. Chemokines provide direction and function for cell movement by attaching to seven transmembrane G-protein-coupled receptors (GPCRS) [44]， and transmitting signals by activating the receptors. Different chemokines play a critical role in regulating the immune system's response. Conventional chemokine receptors bind to their ligands and then communicate by forming connections with G proteins, which also influence cellular migration and other biological processes. While atypical chemokine receptors fail to activate signal transduction upon adhering to their respective ligands, they are vital in maintaining the equilibrium of the chemokine network [45, 46]. Comprising close to 50 chemokine ligands, 20 signaling GPCRs, and 4 ACKRs, the chemokine system is crucial in development, balance, inflammation, infection, and numerous pathological activities, including tumorigenosis. By connecting with Gai-protein-coupled seven-transmembrane-spanning receptors (GPCRs), cellular chemotaxis, adhesion, localization, and cell-to-cell contacts can be controlled. Chemokines have the capability to either directly stimulate or suppress the formation of new blood vessels in tumors, and they also play a role in multiple disease mechanisms as a pathway for signal transmission. Chemokines can also attach to atypical chemokine receptors (ACKRs), which do not attach to G proteins nor initiate chemokine reactions [47].

Chemokines regulate the start, accumulation, emergence, and function of immune cells in the tumor immunological microenvironment (TME) throughout the growth of tumors. Therefore, chemokines and their corresponding receptors play a critical role in promoting the growth of tumors as well as the immune system's response to them [48]. It has been discovered that Chemokine (C-C Motif) Ligand 28 (CCL28) contributes to the emergence of certain gynecological disorders. Known alternatively as mucosa-associated epithelial chemokine (MEC), this protein consists of 127 amino acids. CCL28 possesses the capability to attach to the C-C chemokine receptor 3 (CCR3). And it was discovered that CCR3 is present in white blood cells, including eosinophils, basophils, mast cells, and certain peripheral blood T lymphocytes [49]. CCL28 markedly enhanced the growth and penetration of endometrial stromal cells [50]. The composition and distribution of C-C motif chemokine 28 have not been examined in any studies on cervical cancer to date, nonetheless, this investigation confirmed a strong causation for the disease.

A significant correlation exists between cytokines, chemokines, and serum metabolites. For patients with severe conditions, arginine demonstrated a significant association with inflammatory cytokines, including IL-6, IL-1β, M-CSF, IL-12p70, IFN-α2, and others [51]. Additionally, it has been shown that metabolite lactate signaling systems influence a number of physiological and pathological processes, including cancer, inflammation, and fibrosis. These findings may point the way toward new treatment avenues [52]. These significant metabolic pathways might regulate the release of cytokines and chemokines that promote inflammation. Metabolic reprogramming, as a characteristic hallmark of cancer, is viewed as a key element in driving tumor growth. Furthermore, techniques in metabolomics and proteomic analysis serve as potent instruments for examining disease processes and therapies on a molecular scale, offering fresh insights for forecasting and assessing the effectiveness of drugs. A rise or fall in metabolite levels is regarded as an indicative biomarker, enhancing our comprehension of cancer origins [53]. Metabolite signal transduction is an essential process that affects mesenchymal transformation in epithelial cells, cellular proliferation, cancerous changes, cell differentiation inhibition, and cancer dryness regulation. Several of these metabolites have critical roles in the development of cancer. Tumor growth and development can be accelerated by the ability of cancerous cells to recognize and internalize signals from a variety of metabolites, including lipids, amino acids, nucleotides, and intermediate metabolites in central carbon metabolism [17]. Previous studies have shown a causal relationship between elevated levels of beta-hydroxyisovalerate and myasthenia gravis colon cancer risk [54, 55]. Alpha-hydroxyisovalerate predicts a higher risk of diabetes and is associated with a higher risk of head and neck squamous cell carcinoma (HNSCC) [56, 57]. In this paper, the influence of α-hydroxyisovalerate on cervical cancer is further explained and studied.

The immune system is a complex network of cells, tissues, and organs that defends the body against pathogens and other chemicals. Immune cells release several proteins, such as soluble receptors, chemokines, and cytokines, which have an impact on cell-mediated immune responses [58]. Chemokines, in particular, can change when an infection does, which helps in early identification and predicting whether an HPV infection will result in an immune response to cervical cancer. Women tend to show elevated chemotactic marker levels in CIN2/3 patients [59]. The results of this study on C-C motif chemokine 28 may be useful in the future for the diagnosis and treatment of cervical cancer, based on the analysis shown above. And the result drawn from this research suggests that chemokines can regulate this process through Alpha-hydroxyisovalerate, despite the fact that this interaction has not been well studied.

In this study, we applied the Mendelian randomized design method to explore the causal relationship between inflammatory factors and cervical cancer, as well as the mediating role between inflammatory factors and cervical cancer. Reverse causality is minimized in our Mendelian randomization simulation in order to eliminate confounding bias, prevent interference from reverse causality, and find possible mediators through mediation analysis. Furthermore, a sequence of sensitivity analyses was performed to guarantee the consistency and objectivity of the analysis outcomes. Mendelian randomization (MR) provides distinct benefits in causal analysis, yet it is not without its drawbacks. First, independence and exclusivity may be called into question by the crucial problem of pleiotropy. Instrumental variable bias is an additional issue that can challenge this associational assumption. External elements also play a role in aggravating these difficulties [60, 61]. The diversity within a population can have a nuanced impact on the fundamental premises of MR.

Furthermore, pinpointing the precise processes leading to the disease's development continues to be a complicated task. In addition, the inferences made from the GWAS were based on samples predominantly from Europe, constraining its broader relevance. Attaining the universal applicability needed for MR studies typically necessitates a more extensive sample size. Consequently, although MR serves as an effective method for determining causality, it's crucial that its results are supported by solid evidence from superior randomized controlled trials, guaranteeing a thorough and meticulous method for comprehending intricate biological connections [61].

5. CONCLUSION

To sum up, this study investigated the interaction between metabolites, circulating inflammatory agents, and cervical cancer in detail and found that C-C motif chemokine 28 influences cervical cancer by means of alpha-hydroxyisovalerate. Similar to determining that Alpha-hydroxyisovalerate is a causative mediator in the relationship between cervical cancer and C-C motif chemokine 28. These correlations may still prove to be useful biomarkers and prospective targets in the quest to comprehend the molecular underpinnings of cervical cancer and to create novel treatments.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

(1) The conception and design of the study, or acquisition of data, or analysis and interpretation of data: Cong Xu, Yonghong Xu, Chaowen Chen, Min Wang

(2) Drafting the article or revising it critically for important intellectual content: Cong Xu, Fang Liu

(3) Final approval of the version to be submitted:Guangming Wang

DATA AVAILABILITY STATEMENT

The datasets generated during and analyzed during thecurrent study are publicly available.

ETHICAL STATEMENT

Given that all data originated from online databases, patients' written informed consent was secured. Moreover, our research relied on open-source data, eliminating any pertinent ethical concerns.

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