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Identification of EDNRA as the Key Biomarker for Hypercholesterolemia and Colorectal Cancer

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Some studies have investigated the role of cholesterol in the progression of colorectal cancer (CRC). However, the underlying mechanism of action is not clear. In this study, we used bioinformatics tools to elucidate the molecular mechanisms involved. We initially obtained CRC datasets from the Gene Expression Omnibus (GEO) database and hypercholesterolemia data from GeneCards and DisGeNE. Common differentially expressed genes (DEGs) were determined by using Venn diagram web tools. Next, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The hub gene was identified through common expression pattern analysis and survival analysis. Finally, we conducted an immune regulatory point analysis and predicted target drugs based on the hub gene. The results of our analysis revealed 13 common DEGs, with endothelin receptor type A (*EDNRA*) identified as the hub gene linking hypercholesterolemia and CRC. The results of the GO analysis showed that the common DEGs were primarily associated with the G-protein coupled receptor signaling pathway, extracellular space, and receptor binding. The results of the KEGG pathway enrichment analysis indicated enrichment in pathways related to cancer and the phospholipase D signaling pathway. Additionally, we identified potential target drugs, including *Podocarpus montanus*, *Diospyros kaki*, Herba Salviae japoniae, sitaxentan, and ambrisentan. We found that EDNRA might be an underlying biomarker for both hypercholesterolemia and CRC. The predicted target drugs provide new strategies for treating CRC.

Keywords: biomarker; colorectal cancer; EDNRA; hypercholesterolemia
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Introduction

Colorectal cancer (CRC) ranks as the second most frequent malignancy among women and the third most prevalent among men globally, according to data from Global Cancer Statistics for 2018 (Bray et al. 2018). In 2018, there were approximately 1.8 million new cases and 881,000 fatalities attributed to this disease worldwide (Thanikachalam and Khan 2019). The incidence of CRC is on the rise, particularly among younger individuals. Projections suggest that by 2035, there could be an additional 2-5 million new cases of CRC globally (Arnold et al. 2017; Bray et al. 2018). Despite substantial advances in CRC treatment, it remains the fourth deadliest malignancy worldwide, resulting in approximately 900,000 deaths annually (Dekker et al. 2019). The pathogenesis of CRC remains elusive, necessitating further research to elucidate its precise mechanisms and associated management.

Hypercholesterolemia is defined as having a total cholesterol level exceeding 200 mg/dL (equivalent to > 5.2 mmol/L). In the United States, more than 31 million individuals have total cholesterol levels exceeding 240 mg/dL. These individuals face a two-fold higher risk of atherosclerotic cardiovascular disease (ASCVD) compared to those with normal total cholesterol levels (Karr 2017). With improvements in economic and living conditions, the prevalence of hypercholesterolemia is increasing, posing a greater economic burden on families, society, and nations.

Some studies have reported the detrimental effects of hypercholesterolemia on the immune system's ability to

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monitor tumor cells (Sag et al. 2015). Hypercholesterolemia has been implicated in the progression of various tumors, including breast cancer, prostate cancer, and CRC (Murai 2015; Buss and Dachs 2018; Kim et al. 2021). Guodong Tie's research revealed that elevated cholesterol levels may heighten susceptibility to CRC by reducing the number of NKT and T cells (Tie et al. 2017). However, the mechanism underlying hypercholesterolemia-induced CRC remains unclear. In this study, we investigated the genetic relationship between hypercholesterolemia and CRC using bioinformatics methods commonly employed for identifying potential disease targets and signal pathways for diagnosis and disease prediction (Wan et al. 2020). Initially, we obtained hypercholesterolemia and CRC datasets from the Gene Expression Omnibus (GEO), GeneCards, and DisGeNE databases. Subsequently, we conducted differential gene expression analysis, identified common expression patterns, and performed survival analysis to pinpoint the hub gene. We also conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses to uncover the underlying connections between hypercholesterolemia and CRC. Finally, we conducted an immune regulatory point analysis and predicted target drugs based on the hub gene. A detailed flowchart of the study is provided in Fig. 1.

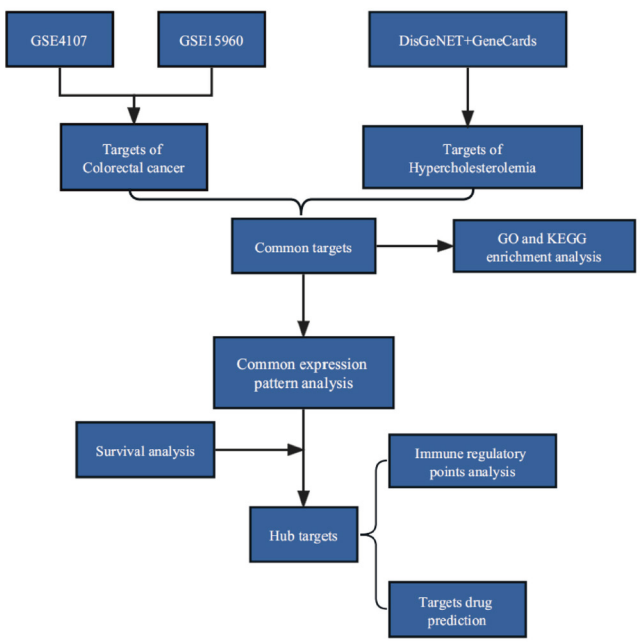

Fig. 1. The flowchart of our study.
First, we obtained the targets of colorectal cancer (CRC) and hypercholesterolemia, respectively. Then, the overlapped targets were identified and were used to carry out the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Next, we perforemed the common expression pattern analysis and survival analysis to identify hub gene. Finally, we performed an immune regulatory point analysis and predicted target drugs.

Materials and Methods

Data collection

The datasets of colorectal cancer (GSE4107 and GSE15960) were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database (Barrett et al. 2013). The datasets of hypercholesterolemia were acquired from the GeneCards (Safran et al. 2010) (https://www.genecards.org/) and DisGeNE (Piñero et al. 2017) (https://www.disgenet.org/) databases. The detailed information is presented in Table 1.

Identification of common targets between CRC and hypercholesterolemia

Based on the GSE4107 and GSE15960 datasets, the common differentially expressed genes (DEGs) of colorectal cancer were obtained using the package 'limma' in the R software (Ritchie et al. 2015). The targets of hypercholesterolemia were obtained by overlapping the targets in the GeneCards and DisGeNE databases. The Venn tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) (Vyas et al. 2009) was implemented to identify the common targets between CRC and hypercholesterolemia, using the above-mentioned data. The GO and KEGG, pathway enrichment analyses, were conducted for the common targets to further investigate the correlation between CRC and hypercholesterolemia.

Identification of the hub gene

Based on the GSE4107 and GSE15960 datasets, a common expression pattern analysis was performed to select the important targets. Survival analysis was conducted based on the significant targets to identify the hub gene using the Kaplan-Meier plotter (https://kmplot.com/analysis/) (Stel et al. 2011).

Immune checkpoint analysis and prediction of target drugs

The immune checkpoint analysis was performed to evaluate the relationship between immune checkpoints and hub genes. The target drugs were predicted using the Drug-Gene Interaction Database (Freshour et al. 2021) (GDIBD, https://dgidb.org/) and the HERB (Fang et al. 2021) (http://herb.ac.cn).

Results

Identification of common targets between CRC and hypercholesterolemia

Based on the GSE4107 and GSE15960 datasets, differential gene expression analyses were performed to select the targets of colorectal cancer. The |logFC| was set as ≥ 1, and the P-value was determined as < 0.05. The volcano plots are shown in Fig. 2A, B. In total, 2,972 genes (1,261 up-regulated genes and 1,711 down-regulated genes) were obtained in the GSE4107 dataset and 4,535 genes (2,175 up-regulated genes and 2,360 down-regulated genes) in the GSE15960 dataset. From these datasets, 986 overlapped

Table 1. The detail information of GSE4107 and GSE15960.

Series	Platform	GeneChip	Samples
GSE4107	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	22
GSE15960	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	12

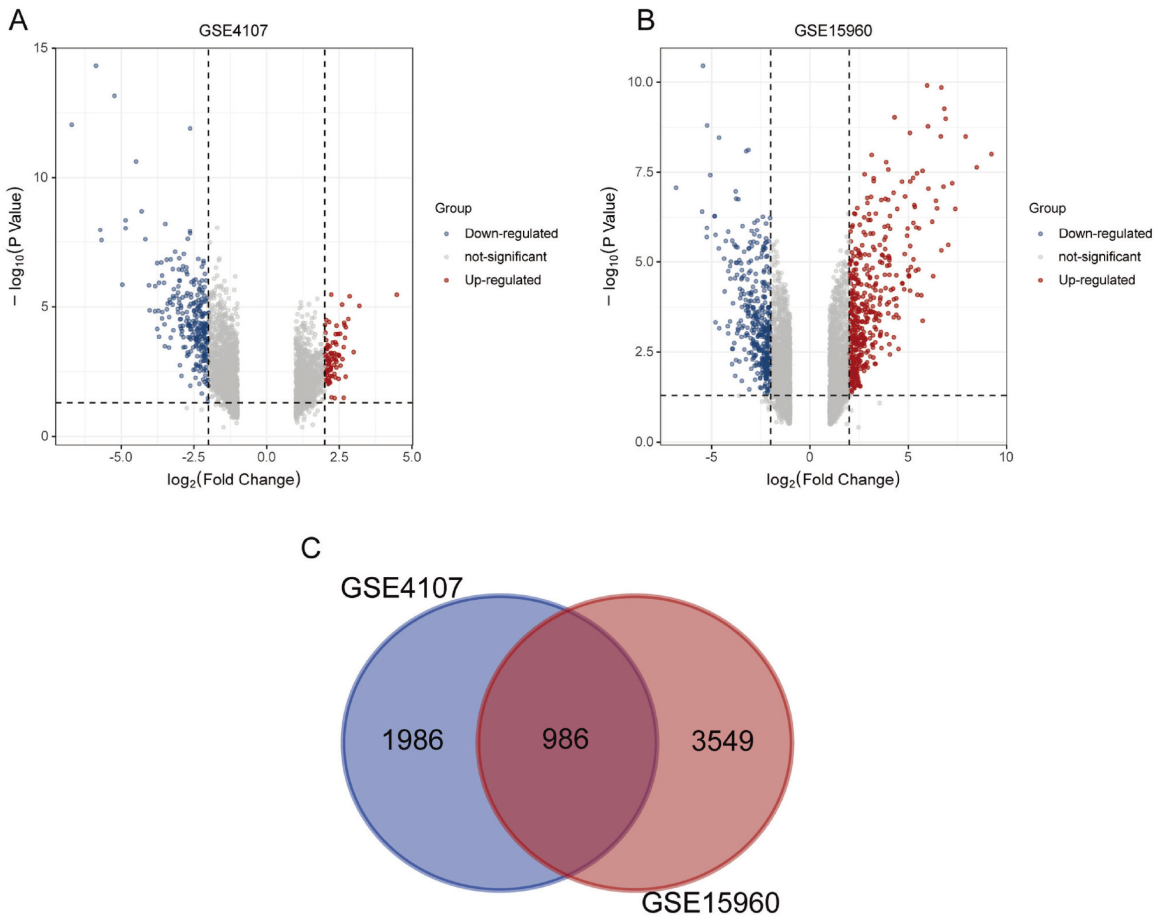

Fig. 2. The identification of colorectal cancer (CRC) targets.
(A) The volcano plot of GSE4107. (B) The volcano plot of GSE15960. (C) The identification of 986 targets in CRC. Red represents up-regulated targets, and blue represents down-regulated targtes.

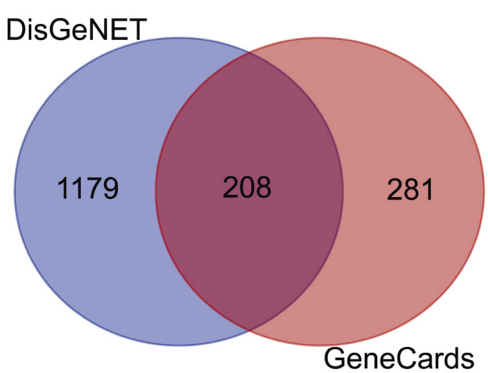

Fig. 3. The identification of 208 targets in hypercholesterolemia. There are 1,387 targets in DisGeNET and 489 targets in GeneCards.

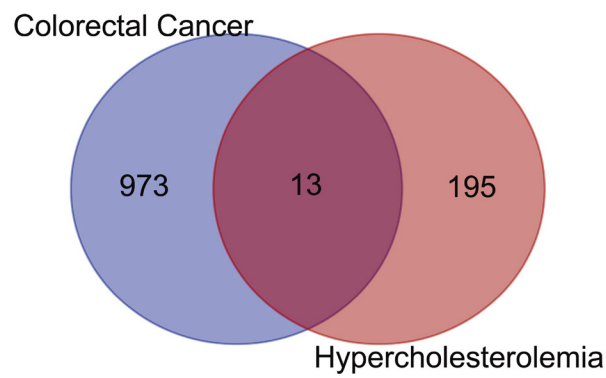

Fig. 4. The identification of 13 common targets between hypercholesterolemia and colorectal cancer (CRC).

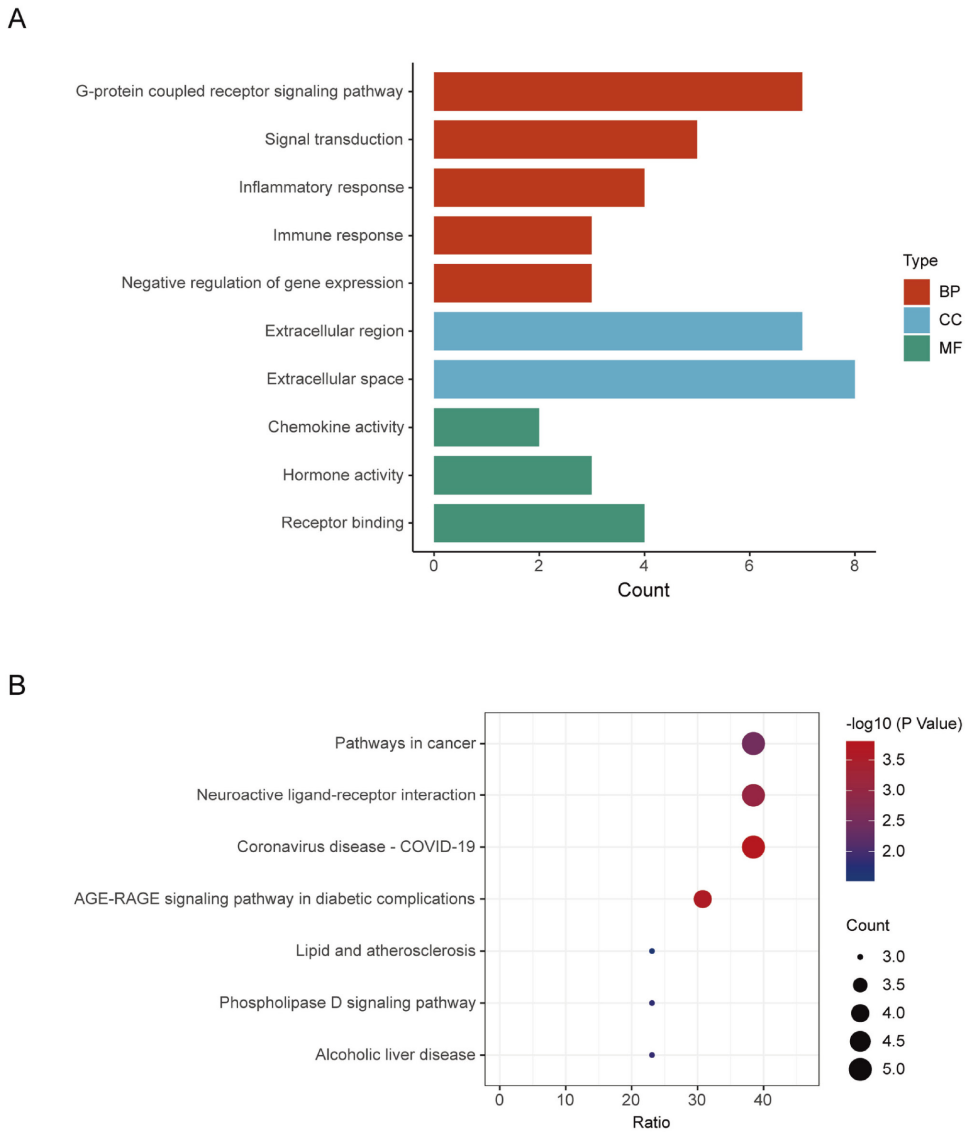

Fig. 5. The results of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses.
(A) The results of GO enrichment analysis. (B) The top 7 results of KEGG pathway analysis. BP, biological process; CC, cellular component; MF, molecular function.

genes were found to be associated with CRC (Fig. 2C). We obtained 1,387 genes in DisGeNE and 489 genes in GeneCards. Among them, 208 genes were found to be associated with hypercholesterolemia (Fig. 3). We identified 13 common targets between CRC and hypercholesterolemia (Fig. 4).

GO and KEGG pathway enrichment analyses

For common targets, GO and KEGG pathway enrichment analyses were performed. The results of the GO analysis showed that the common targets were mostly enriched in the G-protein coupled receptor signaling pathway, signal transduction, inflammatory response, immune response, and negative regulation of gene expression in biological process (BP). The targets were also enriched in the extracellular region and extracellular space in cellular component (CC), and enriched in chemokine activity, hormone activity, and receptor binding in molecular function (MF) (Fig. 5A). The results of the KEGG pathway analysis showed that pathways in cancer, the AGE-RAGE signaling pathway in diabetic complications, and the Phospholipase D signaling pathway were enriched (Fig. 5B).

Identification of the hub gene

The common expression pattern analysis was conducted, and three key genes (*EDNRA*, endothelin receptor type A; *CXCL8*, Interleukin-8; and *ADA*, adenosine deaminase) were selected in the GSE4107 and GSE15960 datas-

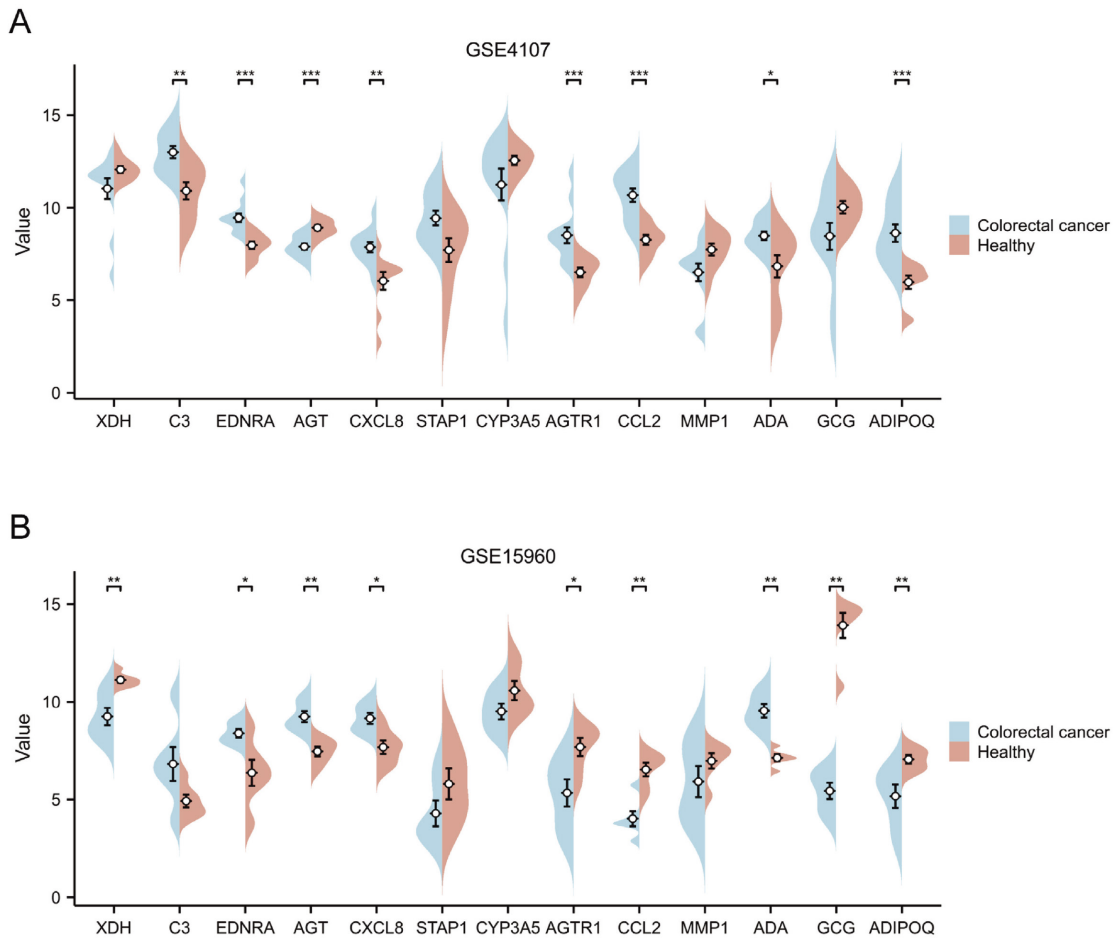

Fig. 6. The identification of 3 key targets (*EDNRA*, endothelin receptor type A; *CXCL8*, Interleukin-8; and *ADA*, adenosine deaminase).
(A) The expression level of 13 common targtes in GSE4107. (B) The expression level of 13 common targtes in GSE15960.

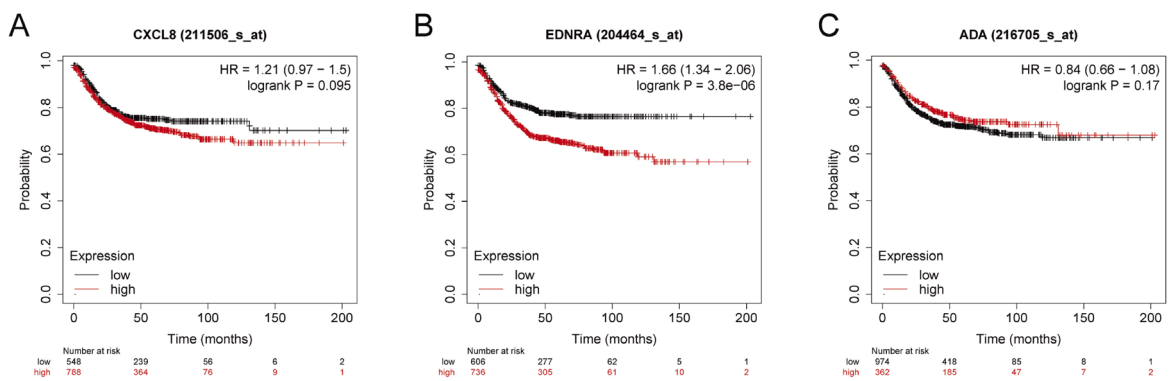

Fig. 7. The identification of hub target, *EDNRA*.
(A) The survival analyis of *CXCL8* ($P > 0.05$). (B) The survival analyis of *EDNRA* ($P < 0.05$). (C) The survival analyis of *ADA* ($P > 0.05$).

ets (Fig. 6). Next, survival analyses were performed to identify *EDNRA* as the hub gene (Fig. 7).

Immune checkpoint analysis and prediction of target drugs

The results of the immune checkpoint analysis showed close relationships between *EDNRA* and several immune

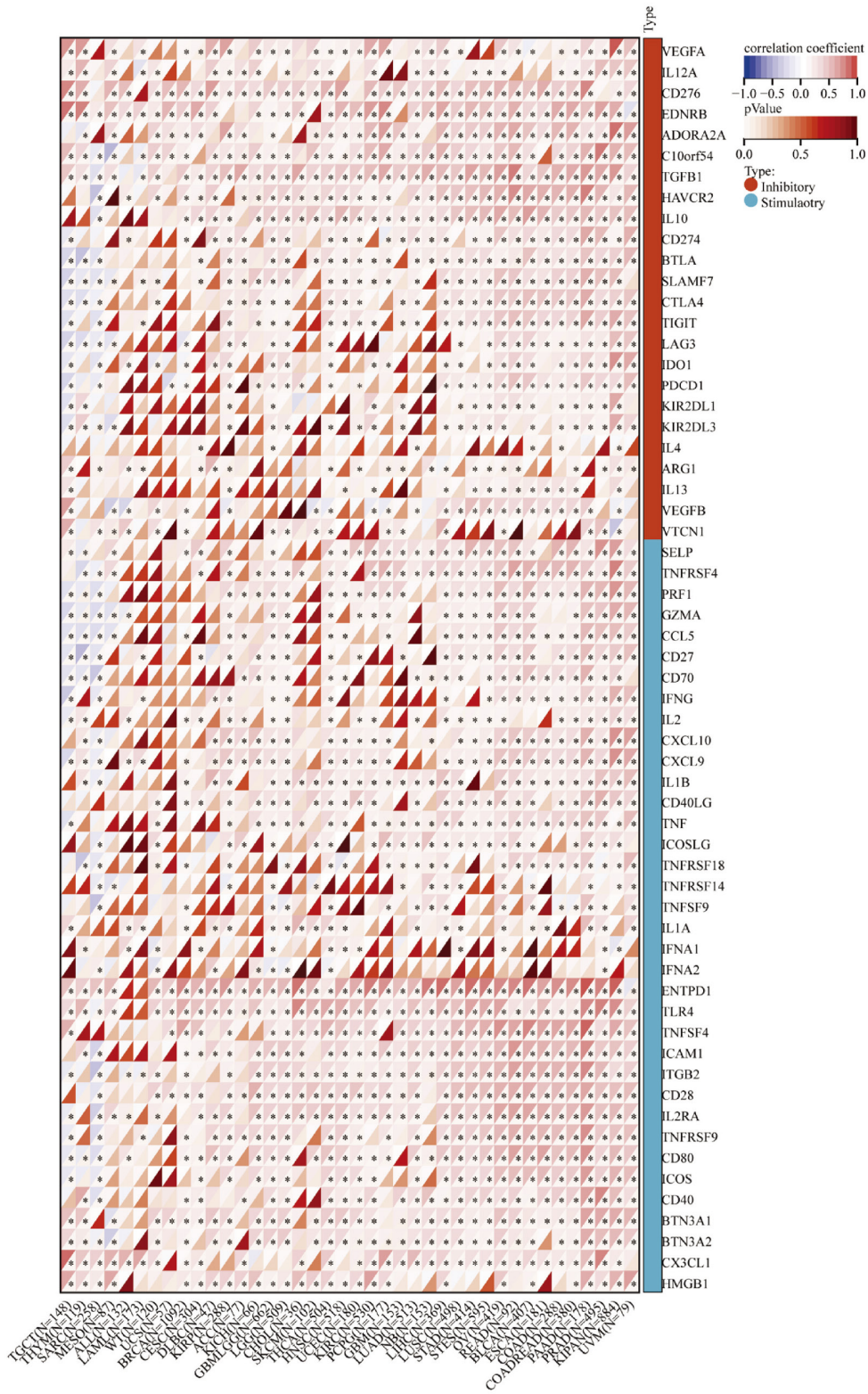

Fig. 8. The results of the immune checkpoint analysis.
In many tumors, EDNRA shows close relationship with many immune regulated targtes.

checkpoint genes (Fig. 8), which indicated that *EDNRA* might be the underlying immune checkpoint in CRC. In the analysis to predict target drugs, sitaxentan, ambrisentan, Podocarpus montanus, Diospyros kaki, Herba Salviae japoniae, Physochlaie Radix, and Fructus Choerospondiatis were discovered in the GDIBD and HERB databases (Table 2).

Table 2. The information of target prediction drugs.

ID	Drug name	P value	Function
CID 216235	Sitaxentan	1.05E-03	Endothelin receptor antagonists
CID6918493	ambrisentan	1.03E-03	Endothelin receptor antagonists
HERB002446	Physochlaie Radix	1.22E-03	Muscarinic receptor antagonist
HERB005135	Herba Salviae japoniae	1.42E-03	Inhibiting cell proliferation
HERB005082	Diospyros kaki	1.42E-03	Muscarinic receptor antagonist
HERB004877	Podocarpus montanus	1.42E-03	Promoting blood circulation and arresting pain

Discussion

CRC ranks as the third most frequently diagnosed cancer and is the second leading cause of cancer-related mortality worldwide, posing challenges in terms of treatment options (Ahmad et al. 2021). The incidence of CRC among younger individuals is on the rise, partly attributable to dietary habits and metabolic disorders (Tie et al. 2017; Thanikachalam and Khan 2019). Hypercholesterolemia is known to increase the risk of CRC and plays a significant role in its progression (Tie et al. 2017). However, the precise underlying mechanism remains unclear. This study employed bioinformatics tools to explore the genetic-level molecular mechanisms and identify potential treatment avenues for CRC.

In this study, the intersection of the GSE4107 and GSE15960 datasets yielded 986 targets associated with CRC. Additionally, 208 genes related to hypercholesterolemia were identified from the GeneCards and DisGeNE databases. To investigate the connection between CRC and hypercholesterolemia, 13 common targets were selected for further analysis through GO and KEGG pathway enrichment studies. GO mainly comprises three parts, including biological process (BP), cellular component (CC), and molecular function (MF) (Kuang and Liu 2022). In BP, the G-protein coupled receptor signaling pathway, signal transduction, inflammatory response, immune response, and negative regulation of gene expression were enriched. In CC, the extracellular region and extracellular space were enriched, indicating a close association with the extracellular microenvironment. In MF, chemokine activity, hormone activity, and receptor binding were enriched. These findings align with previous research, suggesting that hypercholesterolemia may contribute to CRC development by promoting bile acid synthesis and inducing gastrointestinal inflammation responses (Jia et al. 2018). Cholesterol, a primary component in bile acid synthesis, might play a role in CRC carcinogenesis (Axelson et al. 2000). Hypercholesterolemia may participate in the carcinogenesis of CRC by promoting bile acid synthesis. Brindisi et al. (2023) showed that cholesterol can induce the epithelial-mesenchymal transition (EMT) in breast cancer cells by activating the ERRα pathway and generating inflammatory mediators that control the tumor microenvironment. Wei et al. (2019) found that EMT can contribute to metastasis in CRC cells. Hypercholesterolemia may have a role in CRC cell metastasis through the induction of EMT.

In the KEGG pathway, the common targets were mainly enriched in two pathways: the AGE-RAGE signaling pathway in diabetic complications and the Phospholipase D signaling pathway. Advanced glycation end products (AGEs) result from saccharides through the Maillard reaction (Shen et al. 2020). They can increase reactive oxygen species (ROS), proinflammatory cytokines, and reactive nitrogen intermediates (RNIs), leading to inflammation and immunosuppression by binding to receptors for AGE (RAGEs) (Uribarri et al. 2007). The AGE-RAGE signaling pathway inhibits cell proliferation and epithelial-mesenchymal transition in CRC (Yamamoto et al. 2023). Hypercholesterolemia may contribute to CRC development through the AGE-RAGE signaling pathway. Phospholipase D, including PLD1 and PLD2, is a phosphatidylcholine-specific enzyme with therapeutic relevance for several diseases (Scott et al. 2009; Nelson and Frohman 2015). In CRC, the inhibition of phospholipase D1, as found by Hwang et al (2022), leads to the demise of immunogenic cells and enhances cancer immunotherapy by controlling β-catenin signaling. These common targets mainly enrich the Phospholipase D signaling pathway, suggesting its potential as a therapeutic target for CRC.

Common expression pattern analysis and survival analyses were conducted to identify key targets. The results of the common expression pattern analysis revealed significant differences in the expression levels of *EDNRA*, *ADA*, and *CXCL8* between CRC patients and healthy groups in the GSE4107 and GSE15960 datasets. In the survival analyses, only the association of EDNRA with colorectal tumor prognosis reached statistical significance. ADA, a key component of purine metabolism, converts adenosine and deoxyadenosine into inosine and deoxyinosine, respectively, and is widespread across various organisms (Cristalli et al. 2001). Furthermore, it is essential to the development and maturation of the lymphoid system (Cristalli et al. 2001). ADA may be involved in CRC development by regulating the expression of immune cells. CXCL8, initially identified as a chemokine, attracts polymorphonuclear inflammatory leukocyte infiltration by acting on CXCR1/2 and has been implicated in various tumors (Alfaro et al. 2017). CXCL8 contributes to CRC progression through its

pro-tumoral functions, including the regulation of angiogenesis, transmission of survival signaling, and attraction of myeloid cells (Alfaro et al. 2017). EDNRA, a member of the G-protein-coupled receptor family and a marker in pericytes and smooth muscle cells (SMCs), can transform into cancer-associated fibroblasts (Ling et al. 2023). EDNRA regulates various cellular and biological processes by interacting with G proteins, such as signaling target regulation, blood pressure regulation, cell proliferation, cell migration, and cell apoptosis (Bagnato and Natali 2004; Kohan et al. 2011; Rosanò et al. 2013; Rosanò and Bagnato 2016; Bondurand et al. 2018). In CRC, EDNRA promotes cancer cell migration and proliferation while inhibiting apoptosis by interacting with endothelin 1 (EDN1), contributing to CRC progression (Lee et al. 2023). The precise mechanism of its action remains unknown. Based on the results of the KEGG pathway analysis, we hypothesize that EDNRA may drive CRC progression via the AGE-RAGE signaling pathway and Phospholipase D signaling pathway, suggesting its potential as an underlying marker for CRC.

To investigate potential treatment options for CRC, we conducted an immune checkpoint analysis. The results revealed a strong correlation between EDNRA and immune checkpoint targets, suggesting that EDNRA could serve as an immune checkpoint and a potential therapeutic target for CRC. We performed target drug prediction based on EDNRA, which identified several potential target drugs for CRC, including sitaxentan, ambrisentan, Podocarpus montanus, Diospyros kaki, Herba Salviae japoniae, Physochlaie Radix, and Fructus Choerospondiatis. Sitaxentan is a specific antagonist of EDNRA and is commonly used in patients with pulmonary arterial hypertension (Scott 2007; Pulido et al. 2009). Our study highlights the significant role of EDNRA in the development and progression of CRC, suggesting that sitaxentan could be an effective target drug for treating CRC. However, further research is necessary to confirm this hypothesis.

This study has some limitations. Firstly, while our bioinformatics analysis suggests a potential connection between EDNRA, hypercholesterolemia, and CRC, further clinical trials are needed to validate our initial findings. Secondly, the existing research on the association between EDNRA and CRC is limited, emphasizing the need for additional investigations to uncover the mechanisms by which EDNRA contributes to CRC progression.

In conclusion, we used the bioinformatics method in this study and found that EDNRA might be the potential key biomarker and therapeutic target in hypercholesterolemia and CRC. Based on our analysis, we predicted a few potential targeted therapeutic agents that might be used for treating CRC.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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