Validation of quercetin in the treatment of colon cancer with diabetes via network pharmacology and molecular dynamics simulation

Mingqing Wang
First Affiliated Hospital of Anhui Medical University

Guodong Cao
First Affiliated Hospital of Anhui Medical University

Weiguo Zhou
First Affiliated Hospital of Anhui Medical University

Wei Cao
First Affiliated Hospital of Anhui Medical University

Kang Yang
First Affiliated Hospital of Anhui Medical University

Xun Zhang
First Affiliated Hospital of Anhui Medical University

Peng Zhang
First Affiliated Hospital of Anhui Medical University

Zehua Zhang
First Affiliated Hospital of Anhui Medical University

Bo Chen
First Affiliated Hospital of Anhui Medical University

Kongwang Hu
First Affiliated Hospital of Anhui Medical University

Maoming Xiong (ayfyxmm163.com)
First Affiliated Hospital of Anhui Medical University

Research Article

Keywords: Colon cancer, Quercetin, Network pharmacology, Molecular docking, Bioinformatics, Molecular dynamics simulation

Posted Date: January 11th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2458316/v1
License: ☎️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Molecular Diversity on September 25th, 2023. See the published version at https://doi.org/10.1007/s11030-023-10725-4.
Abstract

Objectives

Patients suffering from colon cancer with diabetes (CRC-Diabetes) are more likely to metastasis and relapse when compare with colon cancer (CRC). However, there is a lack of a prognostic model and efficient treatment for CRC-Diabetes. Based on these clinical requirements, this study built a prognosis model for CRC-Diabetes and analyzed whether quercetin could be used for CRC-Diabetes treatment through network pharmacology, Molecular dynamics simulation and bioinformatics.

Methods

Firstly, the differentially expressed genes (DEG) in colon cancer and the related genes in diabetes were screened, and the intersection genes of the two gene clusters were used to construct the prognosis model. Then the potential prognostic markers were screened by univariate Cox proportional hazards regression and lasso regression. Furthermore, multivariate Cox proportional hazards regression was used to construct the prognosis model of CRC-Diabetes. Consequently, quercetin related target genes were screened. The intersection of quercetin target genes with CRC-Diabetes genes was used to find the potential target for quercetin in the treatment of CRC-Diabetes. Molecular docking and molecular dynamics simulation were used to screen reliable targets for quercetin in treatment of CRC-Diabetes.

Results

There are 1008 intersection genes between colon cancer and diabetes. The constructed multivariate Cox proportional hazards regression model based on the above genes shows that the ROC values of 1, 3 and 5 years are 0.787, 0.793 and 0.85 respectively. There are 101 intersection genes in quercetin and CRC-Diabetes. Through molecular docking, seven proteins (HMOX1, ACE, MYC, MMP9, PLAU, MMP3, MMP1) were selected as potential targets of quercetin. We conducted molecular dynamics simulation of quercetin and the above proteins respectively, and found that the binding structure of quercetin with MMP9 and PLAU was relatively stable, which can be considered as a reliable target for quercetin treatment of CRC-Diabetes.

Conclusions

Based on TCGA, TTD, Drugbank and other databases, a prediction model that can effectively predict the prognosis of colon cancer patients with diabetes was constructed. Quercetin can treat colon cancer patients with diabetes by influencing PLAU and its downstream pathways.

Background
Colon cancer is the most common digestive tract tumor and the main cause of death in patients with digestive system tumors. It was reported that in 2020, there were more than 1.9 million new cases of colorectal cancer, and more than 900,000 colorectal cancer patients died, accounting for 10% of the global cancer incidence and death cases\[1\]. However, the relationship between colon cancer and diabetes is still unclear, and the mechanism of interaction is controversial. Feng et al. collected relevant data of 22580 people through NHANES database, showing that diabetes is a high-risk factor for colon cancer\[2\]. Pang et al. recruited 512713 volunteers in the study of the prospective China Kadoorie biobank from 2004 to 2008, and conducted a 10-year follow-up\[3\]. They found that diabetes was a high-risk factor for colon cancer in the Chinese population.

However, a series of experimental researches have revealed that diabetes can promote tumor progression in recent years. If cancer patients complicated with diabetes, the prognosis is often poor\[4; 5\]. Patients with diabetes have special pathology status such as hyperglycemia, hyperinsulinemia, insulin resistance, etc\[6\]. These special physical and chemical states may promote tumor progression\[7\]. Hyperglycemia supplies the tumor cells with large amounts of glucose and enhances the "Warburg effect" (The energy supply of tumor cells mainly depends on the aerobic glycolysis of glucose rather than the oxidative phosphorylation of mitochondria. This effect is called "Warburg effect") to promote tumor proliferation\[6\]. Hyperglycemia also promotes epithelial mesenchymal transition (EMT) via \[8; 9\]. Our previous studies have proved that hyperglycemia can induce mir-26-5p to down regulate the expression of PFKFB3, thereby promoting the EMT of tumors\[10\]. Hyperglycemia can up regulate the expression of integrin αvβ6 by acting on ERK pathway in vitro, and then affect MMP9 and promote liver metastasis of colorectal cancer\[11\]. According to the research of Chang et al, hyperglycemia can promote the proliferation and migration of colorectal cancer by changing the cytoskeleton and inhibiting the functional spectrum of collpasin response mediator protein 2 \[12\]. These studies have proved that hyperglycemia is closely related to the occurrence and progression of cancer. Liang et al. found that AGEs can promote the proliferation and migration of colon cancer cells and reduce tumor cell apoptosis by acting on PI3K/Akt signaling pathway\[13\]. Hyperinsulinemia in patients with diabetes can promote the proliferation and migration of colon cancer. Thien T et al. showed that the proliferation of colon cancer tumors was directly proportional to the dose of intravenous insulin\[14\]. Ruslan novosyadlyy et al. showed that hyperinsulinemia can promote breast tumor proliferation and lung metastasis by up regulating the expression of c-MYC\[15\]. Reducing insulin level can control the metastasis of breast tumor at a low level.

Comprehensively, diabetes may lead to the progression of colon cancer. The incidence rate of diabetes has been high, and the number of colon cancer patients is huge. Therefore, CRC-Diabetes accounts for a large proportion in patients with colon cancer. Relevant retrospective studies found that the treatment efficacy of CRC-Diabetes was poor, and the mortality was significantly higher than that of colon cancer patients without diabetes\[16\]. At present, the treatment of CRC-Diabetes is similar to that of patients with CRC, includes surgery, postoperative chemotherapy, radiotherapy, targeted therapy and immunotherapy\[17\]. The above treatment methods have some limitations, such as high toxicity of chemotherapy drugs, high cost of targeted therapy and immunotherapy. So far, there is a lack of specific strategy for CRC-Diabetes including anti-tumor and anti-diabetes. In recent years, metformin, a
hypoglycemic drug, has also shown its potential in anti-tumor[18]. However, the abuse of metformin may increase the deformity rate of patients' offspring relatives[19; 20]. Some components from natural plants may be the future trend for both anti-tumor and anti diabetes treatment.

Quercetin is a kind of flavonoids, which widely exists in plants, such as berries, apples, onions, tea and so on[21]. In addition, quercetin has anti diabetes, anti-cancer, anti-inflammatory, angiogenesis inhibition, antioxidant and other effects[22–24]. A large number of studies have shown that quercetin can play a therapeutic role in diabetes[25]. Quercetin regulates the absorption of glucose, inhibits the digestion of intestinal carbohydrates, promote isletsβ Cells regeneration and other ways to reduce blood glucose, so as to achieve the treatment of diabetes[25; 26]. At the same time, quercetin also has some anti-tumor effects, mainly through interfering with cell cycle, regulating related signal pathways, inducing tumor cell apoptosis and autophagy, inhibiting angiogenesis, reducing tumor metastasis, reducing drug resistance and so on[27–31]. Angiogenesis is an important factor in tumor volume increase and metastasis.

According to t Xu, X he literature reports, quercetin can inhibit angiogenesis by acting on the expression of MMP2 and MMP9 in colon cancer cells, so as to play an anti-tumor role[32]. Quercetin can inhibit the proliferation and migration of colon cancer cells, and promote the autophagy and apoptosis of colon cancer cells to achieve the purpose of treatment of colon cancer. However, up to now, whether quercetin can play a therapeutic effect on CRC-Diabetes has not been studied, and the related molecular mechanism and their targets of action are still unclear.

Based on the above requirements, this study first constructed a prognosis prediction model for CRC-Diabetes through bioinformatics. By integrating bioinformatics and network pharmacology, we find the target of quercetin and analyze its antitumor molecular mechanism and pathway. Molecular docking screening potential drug binding targets provides a theoretical basis for quercetin from natural ingredients to treat CRC-Diabetes(Figure 1).

**Materials And Methods**

**Identification Of Crc-diabetes Related Genes**

To identify CRC-Diabetes related genes, transcriptome profiles of CRC patients were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer) on January 10, 2022. The differential genes in CRC were screened and obtained using the ‘limma’ package of R-language Bioconductor with pvalue < 0.05 and | logfold change(FC) | >1.

Furthermore, genes related to Diabetes were selected from the GeneCard database, OMIM database, DrugBank database, PharmGkb database and TTD database. Finally, the overlapping targets were obtained and regarded as the CRC-Diabetes related genes by taking the intersection of CRC and diabetes genes.

**Clinical Analysis Of Crc And Diabetes Related Genes**
The 'survival' package in R package was used to analyze the correlation of CRC-Diabetes relates genes and survival time in patients with CRC-Diabetes. Univariate Cox proportional hazards regression was used for prognostic analysis. The genes after univariate Cox analysis were screened by lasso regression. Then, using the screened genes, a multivariable Cox proportional hazards regression model was constructed. The patients were divided into low-risk group and high-risk group according to the average risk score. Finally, R language was used to analyze the clinical characteristics between genes in multivariate Cox proportional hazards regression model and CRC-Diabetes patients.

**Acquisition Of Quercetin Pharmacological Targets In Crc-diabetes**

All quercetin pharmacological targets were collected and screened through accessible online tools such as TCM Systems Pharmacology Database and Analysis Platform (TCMSP), Swiss target prediction and Pharmmapper. Then, use retrieve/ID mapping in UniProt database and select human setting for data correction. Finally, the corrected quercetin target was intersected with CRC-Diabetes related genes to obtain the candidate genes for quercetin treatment of CRC-Diabetes.

**Enrichment Analysis And Network Visualization**

The R language pack, contain "clusterprofiler", "org. HS. eg.db" and "enrichplot", was used to enrich, analyzed and visualized the gene ontology (GO) and KEGG pathway of CRC-Diabetes and quercetin nested genes. Set p-value cutoff = 0.05 and Q-value cutoff = 0.05, obtained GO data through "org. HS. eg.db" enrichment, and draw bubble chart, bar chart and Circos chart with output. The drug-target-GO function-pathway-disease was constructed using Cytoscape software (version 3.82) to visualize the targeting effect and mechanism of quercetin on CRC and diabetes cross genes.

**Identify The Core Target Of Quercetin Against Crc-diabetes**

The overlapping targets of quercetin in the treatment of CRC-Diabetes were queried in the String database, and the protein-protein interaction (PPI) network diagram and TSV data were obtained. Using cytonca in Cytoscape software, select the scores of betweenness, closeness, degree, eigenvector, lac and network to screen out the core genes. The core genes were enrichment analyzed, the GO and KEGG pathways were visualized.

**Molecular Docking**

By molecular docking analysis, the binding ability and binding mode of proteins to small molecule ligands can be predicted. Download the SDF molecular structure of quercetin in PubChem (https://www.rcsb.org/) database. Download the protein structure of CRC-Diabetes related genes in PDB (https://www.rcsb.org/) database. By using the chembio3D draw module in the chembiooooffice software,
the mm2 force field of quercetin was optimized to generate the minimum energy structure and converted it into mol2 format. PyMOL (2.5.1) was used to operate the quercetin structure with the remove solvent and remove organic commands, and Autodock tolls was used to hydrogenate and charge the protein to convert the protein and ligand into the pdbqt format required for molecular docking. Autodock Vina software was used to dock proteins and ligands, and then visually express the docking results through PyMOL (2.5.1), showing the possible binding sites, binding modes and interaction forces between quercetin and proteins.

**Molecular Dynamics Simulation**

First, construct the GAFF position of quercetin through sobtop software, and then construct the charmm36 position of macromolecular protein through gromacs software. Gromacs software was used to add hydrogen atoms to the molecular dynamics simulation system to build a TIP3P solvent box, and Na⁺/Cl⁻ was added to the system to balance the system charge. The NVT (isothermal isomer) system was simulated at 310K to further distribute the solvent molecules uniformly in the solvent box. The equilibrium simulation was carried out under NPT (isothermal isobaric). Finally, molecular dynamics simulation of 100ns.

**Immunohistochemistry Of Colon Cancer Tissue**

Through the online website THE HUMAN PROTEIN ATLAS (http://www.proteinatlas.org/humanproteome). Obtaining the expression of MMP9 protein in colon cancer tissues and normal colon tissues.

**Results**

**Screening Crc-diabetes Targets**

First, 4439 genes related to diabetes were collected from Genecard, OMIM, DrugBank, TTD and PharmGKB online databases (Fig. 2A). 5272 differentially expressed genes related to CRC were screened by TCGA database (Fig. 2B, Supplementary Fig. 1). The genes of diabetes and CRC were intersected, and a total of 1008 intersected genes were obtained (Fig. 2B).

**Construction Of Prediction Model Based On Crc-diabetes Intersection Gene**

In order to construct the prognosis prediction model for CRC-Diabetes patients, 1008 cross genes were analyzed by univariate Cox proportional analysis regression, Lasso regression and multivariate Cox proportional risk regression model. Firstly, 67 candidate genes were identified by univariate Cox analysis. After screening by lasso regression, 29 candidate genes were obtained (Supplementary Fig. 2). Finally, 18
prognostic genes were identified by multivariate Cox proportional hazards regression model, including CAV1, GSTM1, UTS2, CPT2, MIR200B, RP9, CLDN9, WDR72, ENPP2, MAT1A, CD19, MIR148A, SPTBN5, SYCE2, TNNT1, PLSCR3, CALB2, FOLR1 (Fig. 2C, Table 1). In addition, based on the coef value of multivariate Cox proportional hazards regression analysis representing patient risk, we divided patients into high-risk and low-risk groups. Next, in the overall survival analysis, there were significant differences between the high-risk group and the low-risk group (Fig. 3A). The risk score, survival status and the expression distribution of the above 18 genes of each CRC patient were further analyzed. The results showed that the greater the risk value of the patient, the higher the risk score(Fig. 3B). In addition, the clinical prognosis analysis of these 18 genes showed that WDR72, CPT2, MIR200B, MIR148A, CLDN9, FOLR1 and CALB2 were related to distant metastasis(Fig. 4C), and the expression of CALB2, SPTBN5, CPT2 and TNNT1 was closely related to the late stage of CRC (Fig. 4D). However, the expression of CPT2 in CRC stage I and II was higher than that in CRC stage III and IV (Fig. 4A). The expressions of CALB2, RP9, CPT2, MIR148A, CLDN9, FOLR1 and MIR200B were related to the number and range of lymph node metastasis (Fig. 4B). In addition, the expression of SYCE2 and MAT1A in elderly patients was higher than that in young patients (Fig. 4E), the expression of FOLR1 in young patients was higher than that in elderly patients, and the expression of CAV1 in female patients was higher than that in male patients (Fig. 4F).

### Table 1
<table>
<thead>
<tr>
<th>Symbol</th>
<th>coef</th>
<th>HR</th>
<th>HR95L</th>
<th>HR95H</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAV1</td>
<td>0.01230179</td>
<td>1.01237776</td>
<td>1.000210852</td>
<td>1.02692687</td>
<td>0.046136767</td>
</tr>
<tr>
<td>GSTM1</td>
<td>0.063308138</td>
<td>1.063535065</td>
<td>1.007312344</td>
<td>1.126742287</td>
<td>0.0267698</td>
</tr>
<tr>
<td>UTS2</td>
<td>0.177076518</td>
<td>1.193722431</td>
<td>1.003679915</td>
<td>1.419748687</td>
<td>0.045340535</td>
</tr>
<tr>
<td>CPT2</td>
<td>-0.082646179</td>
<td>0.920676844</td>
<td>0.865240265</td>
<td>0.982731921</td>
<td>0.01301453</td>
</tr>
<tr>
<td>MIR200B</td>
<td>0.048148553</td>
<td>1.049326524</td>
<td>1.014089617</td>
<td>1.08578782</td>
<td>0.005730931</td>
</tr>
<tr>
<td>RP9</td>
<td>0.144642548</td>
<td>1.155626416</td>
<td>1.031410399</td>
<td>1.294802161</td>
<td>0.012666325</td>
</tr>
<tr>
<td>CLDN9</td>
<td>0.115310372</td>
<td>1.122216189</td>
<td>0.997073807</td>
<td>1.26307528</td>
<td>0.055954709</td>
</tr>
<tr>
<td>WDR72</td>
<td>0.062308344</td>
<td>1.064290462</td>
<td>1.003772853</td>
<td>1.128456686</td>
<td>0.036975116</td>
</tr>
<tr>
<td>ENPP2</td>
<td>0.072670858</td>
<td>1.075376518</td>
<td>1.032625673</td>
<td>1.119897255</td>
<td>0.00044623</td>
</tr>
<tr>
<td>MAT1A</td>
<td>0.131654645</td>
<td>1.204608532</td>
<td>1.114143319</td>
<td>1.304192259</td>
<td>2.96E-06</td>
</tr>
<tr>
<td>CD19</td>
<td>0.137004343</td>
<td>1.131676808</td>
<td>1.018043418</td>
<td>1.257993889</td>
<td>0.021925668</td>
</tr>
<tr>
<td>MIR148A</td>
<td>0.147648148</td>
<td>1.159104991</td>
<td>0.994265449</td>
<td>1.351273327</td>
<td>0.059229845</td>
</tr>
<tr>
<td>SPTBN5</td>
<td>0.124982079</td>
<td>1.133128146</td>
<td>0.985938313</td>
<td>1.302290253</td>
<td>0.078325746</td>
</tr>
<tr>
<td>SYCE2</td>
<td>0.388698182</td>
<td>1.475047485</td>
<td>1.205105047</td>
<td>1.805388735</td>
<td>0.000163392</td>
</tr>
<tr>
<td>TNNT1</td>
<td>0.045499843</td>
<td>1.04655084</td>
<td>1.001893732</td>
<td>1.083456677</td>
<td>0.010994769</td>
</tr>
<tr>
<td>PLSCR3</td>
<td>1.518779535</td>
<td>4.566631918</td>
<td>1.120843577</td>
<td>18.6057459</td>
<td>0.034078507</td>
</tr>
<tr>
<td>CALB2</td>
<td>0.028813245</td>
<td>1.029232363</td>
<td>0.996627925</td>
<td>1.06330217</td>
<td>0.083149106</td>
</tr>
<tr>
<td>FOLR1</td>
<td>0.026079613</td>
<td>1.026422662</td>
<td>1.015830766</td>
<td>1.037124997</td>
<td>8.32E-07</td>
</tr>
</tbody>
</table>

Coef: Coefficient of multivariate cox proportional risk regression model
Preliminary Screening Of Potential Therapeutic Targets Of Quercetin On Crc-diabetes

The pharmacological targets of quercetin were determined through TCMSP database, Pharmmappe database and Swiss Target Prediction online website. After biological correction and repeated gene deletion using UniProt database, 449 quercetin related target genes were obtained (Fig. 5A). Taking the above CRC-Diabetes intersection genes and quercetin related targets for further intersection, 101 potential target genes that may be quercetin for the treatment of CRC-Diabetes were obtained (Fig. 5B). GO and KEGG enrichment analysis of 101 cross genes showed that quercetin may play a role by affecting the activation of the following pathways, mainly including response to toxic substance, extracellular matrix disassembly, response to hypoxia, cellular response to chemical stress, response to decreased oxygen levels, response to oxygen levels, response to drug, collagen catabolic process, response to reactive oxygen species, response to nutrient levels (Fig. 5D). In addition, in KEGG, there are 52 KEGG pathways related to the target (p < 0.05), including Drug metabolism- cytochrome P450, Metabolism of xenobiotics by cytochrome P450, Nitrogen metabolism, Cellular senescence, Cell cycle, Renin-angiotensin system, Chemical carcinogenesis - reactive oxygen species, Apoptosis - multiple species, Chemical carcinogenesis - receptor activation, Lipid and atherosclerosis, Tyrosine metabolism, Tryptophan metabolism, IL-17 signaling pathway, p53 signaling pathway, TNF signaling pathway, AGE-RAGE signaling pathway in diabetic complications, FoxO signaling pathway (Fig. 5C).

Screening The Core Targets Of Quercetin In The Treatment Of Crc-diabetes

The above 101 intersection genes were mapped into PPI networking by STRING. Input the PPI network TSV file downloaded from STRING into Cytoscape software, and use CytoNCA to calculate the Betweenness, Closeness, Degree, Eigenvector, LAC and Network scores of quercetin anti CRC-Diabetes gene. Eight core gene targets were identified, including HMOX1, ACE, MYC, MMP9, PLAU, CCND1, MMP3, MMP1. GO and KEGG analysis were performed on eight core targets (Fig. 6), and 37 KEGG pathways were obtained (p-adjust < 0.05), including Transcriptional misregulation in cancer, Proteoglycans in cancer, IL-17 signaling pathway, Colorectal cancer, Endocrine resistance, Cell cycle, Relaxin signaling pathway, Cellular senescence, Hippo signaling pathway, JAK-STAT signaling pathway, Wnt signaling pathway, Diabetic cardiomyopathy, Renin-angiotensin system.

Molecular Docking Identified The Target Genes And Binding Sites Of Quercetin On Crc-diabetes

Through molecular docking, the binding sites between quercetin and eight core targets (HMOX1, ACE, MYC, MMP9, PLAU, CCND1, MMP3, MMP1) for the treatment of CRC-Diabetes were simulated and analyzed. It was found that except CCND1 could not bind to quercetin, other core genes could bind to quercetin (Fig. 7A, Fig. 7B, Table 2). The results suggest that quercetin may affect the three-dimensional structure and function of its protein by binding with HMOX1, ACE, MYC, MMP9, PLAU, MMP3 and MMP1,
and further affect its downstream pathway, so as to play an anti-tumor and anti diabetes role against CRC-Diabetes. Quercetin forms hydrogen bond interaction with GLN-28, TYR-520, HIS-513, ASP-453, LYS-454, GLU-384 residues of ACE (1o86 2.00 Å) (Supplementary Fig. 3A); It forms hydrogen bond interaction with THR-135 residue of HMOX1 (1n45 1.50 Å) (Supplementary Fig. 3B); It forms hydrogen bond interaction with ARG-214 residue of MMP1 (1cge 1.90 Å) (Fig. 7E); It forms hydrogen bond interaction with ALA-665, LEU-664, GLU-702 and TYR-720 residues of MMP3 (1hy7 1.50 Å) (Fig. 7D); It forms hydrogen bond interaction with GLN-227 and ALA-189 residues of MMP9 (6esm 1.10 Å) (Fig. 7C); It forms hydrogen bond interaction with SER-224 and ARG-214 residues of MYC (6g6k 1.35 Å) (Supplementary Fig. 3C); It forms hydrogen bond interaction with SER-214, SER-195 and SER-190 residues of PLAU (5yc6 1.18 Å) (Supplementary Fig. 3D). Molecular docking shows that quercetin can form hydrogen bonds with the amino acid residues of these proteins, which proves that quercetin may bind to these proteins and then interact. Comparing the binding ability of these quercetin with the above seven proteins, it was found that the binding ability of quercetin and MMP9 was the strongest. It is suggested that MMP9 may be the main target of quercetin in the treatment of CRC-Diabetes.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding site of quercetin to the protein expression product of quercetin anti-CRC-Diabetes core genes.</td>
</tr>
<tr>
<td>1o86:ACE</td>
</tr>
<tr>
<td>1n45:HMOX1</td>
</tr>
<tr>
<td>1cge:MMP1</td>
</tr>
<tr>
<td>1hy7:MMP3</td>
</tr>
<tr>
<td>6esm:MMP9</td>
</tr>
<tr>
<td>6g6k:MYC</td>
</tr>
<tr>
<td>5yc6:PLAU</td>
</tr>
</tbody>
</table>
Molecular Dynamics Results

The root mean square deviation of molecular dynamics simulation can reflect the mobility of small ligand molecules, while larger RMSD and stronger fluctuations indicate strong mobility. The simulation results show that the RMSD fluctuation of the docking structure of quercetin and PLAU is small, and the mobility of the system is small. MMP9 fluctuates more stably after 10 ns (Fig. 8). The hydrogen bond results showed that the number of hydrogen bonds between quercetin and MMP9 was 2–3. The number of hydrogen bonds between quercetin and PLAU is 1–2 (figure 9).

Immunohistochemistry

According to the immunohistochemical results, we found that the expression of MMP9 and PLAU protein in colon cancer tissues was significantly higher than that in normal colon tissues (Fig. 10). MMP9 and PLAU protein was up-regulated in colon cancer tissues.

Discussion

CRC is the third most common cancer and one of the leading causes of cancer death worldwide. In recent years, with the change of diet, work and rest, the incidence rate of diabetes remains high. According to statistics, there were nearly 1 billion people with diabetes worldwide in 2019, an increase of 62% over 10
years ago[33]. Due to the large number of patients with diabetes and its numerous complications, diabetes has become the top ten leading cause of death among contemporary people[33]. Due to the large number of patients with colon cancer, the total number of CRC-Diabetes cannot be ignored. Diabetes patients with hyperglycemia, hyperinsulinemia, insulin resistance and other special states. These special physical and chemical states often affect the changes of tumor behavior of tumor cells. Studies have proved that diabetes provides a large amount of glucose through hyperglycemia, enhances the level of glycolysis, and promotes the malignant transformation of colon cancer[34]. Therefore, the prognosis of CRC-Diabetes patients is worse, the survival rate is lower, and the probability of tumor recurrence and metastasis is greater. In previous studies, our research team has proved that gastric cancer cells have stronger proliferation and migration ability in high glucose environment[35]. At present, there is a lack of special treatment for CRC-Diabetes (the treatment method is similar to simple colon cancer), which makes the treatment effect of these patients very unsatisfactory. It is urgent to explore an integrated treatment scheme that takes into account both anti-tumor and anti-diabetes.

The predecessor of many antitumor drugs is natural ingredients from nature, such as vincristine, paclitaxel, curcumin, colchicine, lycopene and so on. According to the statistics of antitumor drugs from 1950 to 2010, 48.6% of anticancer drugs are from natural products[36]. Therefore, the components of natural plants are mostly the main source of drugs for the treatment of diseases. In the anti-tumor related treatment, there are still plans to treat tumors with plant ingredients and traditional Chinese medicine. Quercetin is a natural product of flavonoids. In previous papers, quercetin has anti colon cancer effect on colon cancer cells by promoting apoptosis and inhibiting cell cycle[37–39]. Therefore, we speculate that quercetin may have a certain degree of therapeutic effect in CRC-Diabetes.

Based on current demand: 1. Lack of prognosis prediction model for CRC-Diabetes patients; 2. Whether quercetin can play a specific antitumor effect in CRC-Diabetes patients.

So in this study, we first build a prediction model. By screening the differentially expressed genes in colon cancer (4439) and diabetes related genes (5272), 1008 intersection genes between colon cancer and diabetes were obtained. After univariate Cox analysis and lasso regression screening, the multivariate Cox risk proportional regression model was constructed, and the Cox model containing 18 important genes was inferred, including CAV1, GSTM1, UTS2, CPT2, MIR200B, RP9, CLDN9, WDR72, ENPP2, MAT1A, CD19, MIR148A, SPTBN5, SYCE2, TNNT1, PLSCR3, CALB2 and FOLR1. The constructed prognosis model can provide a reference scheme for the prognosis and life cycle evaluation of CRC-Diabetes patients. The clinical correlation analysis of these genes showed that they were related to Age, Gender, T, N, M and tumor Stage.

Through the online data website of traditional Chinese medicine, we screened 449 quercetin genes and selected their intersection genes with colon cancer and diabetes. Eight core genes for quercetin treatment of CRC-Diabetes were screened by Cytoscape, including HMOX1, ACE, MYC, MMP9, PLAU, CCND1, MMP3 and MMP1. Yi-Heng et al. found that MYC can promote the proliferation and growth of colon cancer by inducing LEF1 and activating Wnt pathway[40]. The down-regulation of MYC expression can inhibit the
proliferation and metastasis of colon cancer[41; 42]. Matrix Metalloproteinase (MMP) can interfere with tumor growth by degrading various components in tumor microenvironment, producing active fragments and promoting the release of growth factors. It has protective and inhibitory effects on colon cancer cells[43]. The patients with high expression of MMP1, MMP3 and MMP9 have short survival time and poor prognosis[44; 45]. MMP1 promotes the proliferation, growth, migration and angiogenesis of colon cancer cells by acting on EMT and PI3K/Akt/c-MYC signaling pathway[46]. MMP9 acts on MKK-3/p38/NF-κB cancer promoting pathway leads to advanced metastasis of cancer[47]. KEGG enrichment analysis showed that quercetin treated CRC-Diabetes through the regulation of apoptosis, cell cycle, tumor metabolism and other related signal pathways, including IL-17 signaling pathway, p53 signaling pathway, TNF signaling pathway, AGE-RAGE signaling pathway and Colorectal cancer. Some studies have also shown that the development of colon cancer with type 2 diabetes is mainly due to the activation of ERK1 / 2 and JNK MAPK signaling by insulin / IGF-1[48].

Then, molecular docking was carried out for the above eight core intersection genes (HMOX1, ACE, MYC, MMP9, PLAU, CCND1, MMP3, MMP1). The results suggest that quercetin can produce hydrogen bonds with HMOX1, ACE, MYC, MMP9, PLAU, MMP3 and MMP1. Therefore, these seven genes may be the potential targets of quercetin in the treatment of CRC-Diabetes. By comparing the binding ability of quercetin with these seven genes, it was found that MMP9 had the strongest binding ability with quercetin. According to the results of molecular dynamics simulation, the docking structure of quercetin with MMP9 and PLAU is relatively stable, which may be a reliable therapeutic target for quercetin in the treatment of CRC-Diabetes. It is worth noting that previous studies have suggested that MMP9 plays an important role in the invasion and metastasis of colon cancer, and has a potential impact on the prognosis of colon cancer. Huiyu et al. found that TIPE/MMP-9 acts on MKK-3/p38/NF-κB. To promote the invasion and migration of colon cancer[47]. Shiro et al. showed that the protein level expression of MMP9 increased in hyperglycemic environment, which may be due to the increased oxidative stress caused by hyperglycemic environment, thus promoting the increased expression of MMP9[49]. In this study, immunohistochemical results showed that the expression of MMP9 and PLAU protein in colon cancer tissues was significantly higher than that in normal colon tissues.

**Conclusions**

To sum up, we constructed a prediction model based on 18 differential genes through univariate and multivariate Cox proportional regression model, which can be used to predict the prognostic risk of CRC-Diabetes. It provides a new reference scheme for the prognosis evaluation of CRC-Diabetes patients. Then, by integrating network pharmacology and bioinformatics, the intersection genes of quercetin, colon cancer and diabetes (the target genes of quercetin acting on CRC-Diabetes) were screened out. Based on GO and KEGG enrichment analysis, we obtained the molecular mechanism and signal pathway of quercetin acting on CRC-Diabetes (Fig. 11). The results suggest that quercetin exerts its antitumor effect on CRC-Diabetes patients mainly by affecting cell metabolism, cell cycle and apoptosis. Subsequently, we screened the core genes of quercetin in the treatment of CRC-Diabetes. We found that MMPs may be the main target genes for the treatment of CRC-Diabetes. Quercetin can inhibit tumor angiogenesis and
metastasis by acting on MMP1, MMP3 and MMP9. Through molecular docking and molecular dynamics simulation, it was determined that MMP9, PLAU and quercetin combined stably, which may be the main target of quercetin in the treatment of colon cancer with diabetes.

Declarations

Authors’ contributions

Mingqing Wang, Guodong Cao, Wei Cao, Maoming Xiong, Bo Chen, and Kongwang Hu designed the idea of the article. Weiguo Zhou, zehua Zhang and Peng Zhang download the data. Mingqing Wang and Kang Yang conducted bioinformatics analysis of data. Weiguo Zhou and Mingqing Wang conducted molecular docking. Mingqing Wang wrote the manuscript.

Acknowledgments

This item is not applicable

Funding

This work was supported by Anhui Provincial Natural Science Foundation No.:2208085MH240 .Quality Engineering Project of Anhui Province No.:2020jyxm0898 No.:2020jyxm0910 .Clinical research project of Anhui Medical University No.:2020xkj176 .Soft health science research of Anhui province No.:2020WR01003

References


35. X Xu, B Chen, S Zhu, J Zhang, X He, G Cao, B Chen (2019) Cancer Cell Int. 19: 344.

Figures
Figure 1

Figure 2

Screening gene. (A) Diabetes related genes. (B) Intersect gene of diabetes and CRC. (C) Multivariate Cox proportional hazards regression analysis identified 18 genes. CRC: Colon cancer CRC-Diabetes Genes: Colon cancer with Diabetes
Figure 3

Prognostic value of CRC-Diabetes related genes. (A) Survival curve of high risk group and low risk group. (B) (B1) Analysis of patients’ risk score using Cox proportional hazards regression. (B2) Relationship between risk score and patient survival. (B3) Gene expression level. (C) Multivariate Cox proportional hazards regression model 1, 3 and 5-year ROC curve. CRC-Diabetes Genes: Colon cancer with Diabetes.
Figure 4

Clinical prognostic analysis of 18 genes. (A) Relationship between gene expression and CRC stage. (B) Relationship between gene expression and lymph node metastasis. (C) Relationship between gene expression and distant metastasis of tumor. (D) Relationship between gene expression and depth of tumor invasion. (E) Relationship between gene expression and age of CRC patients. (F) Relationship between gene expression and sex in CRC patients. CRC: Colon cancer
Figure 5

Figure 6

Protein interaction network (PPI) of quercetin anti CRC-Diabetes gene. The core genes of quercetin anti CRC-Diabetes and their protein interaction networks were screened by Cytoscape. CRC-Diabetes Genes: Colon cancer with Diabetes.
Figure 7

Molecular docking of quercetin with its anti CRC-Diabetes core target. (A) Two dimensional structure of Quercetin. (B) Three dimensional structure of Quercetin. (C) Molecular docking of quercetin with MMP9 (6esm). (D) Molecular docking of quercetin with PLAU (5yc6). (E) Molecular docking of quercetin with MMP1 (1cge). CRC-Diabetes Genes: Colon cancer with Diabetes
root mean square deviation (RMSD) difference over time. (A) Rmsd of ligands and all proteins (B) Ligand and PLA\'s RMSD (C) Ligand and MMP9\'s RMSD (D) Ligand and ACE\'s RMSD (E) Ligand and HMOX1\'s RMSD (F) Ligand and MMP1\'s RMSD (G) Ligand and MMP3\'s RMSD (H) Ligand and MYC\'s RMSD
Figure 9

Hydrogen bond analysis. (A) ACE Hydrogen bond analysis (B) HMOX1 Hydrogen bond analysis (C) MMP1 Hydrogen bond analysis (D) MMP9 Hydrogen bond analysis (E) MYC Hydrogen bond analysis (F) PLAU Hydrogen bond analysis

Figure 10

Figure 11

Interaction network of quercetin anti CRC-Diabetes drug action target, drug action pathway and drug action mechanism. CRC-Diabetes Genes: Colon cancer with Diabetes

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Caption.docx
- SupplementaryFigure1.tif
- SupplementaryFigure2.tif
• SupplementaryFigure3.tif