High Expression of Mitochondrial Autophagy-Related Gene FUNDC1 is Associated with Poor Prognosis of Colon Cancer and Defective Immune Cell Infiltration

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Research Article

Keywords: Mitochondrial autophagy, Colon adenocarcinoma, FUNDC1, Immune cell infiltration, Prognosis

DOI: https://doi.org/10.21203/rs.3.rs-705751/v1

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Abstract

Background:

Mitochondrial dysfunction is related to the occurrence and development of many diseases. FUNDC1 has attracted attention as a receptor related to mitochondrial autophagy. Colorectal cancer is the fourth most commonly diagnosed cancer. This article uses bioinformatics analysis to study the relationship between FUNDC1 and immune cell infiltration and prognosis of colon cancer.

Methods:

The RNA sequencing data and detailed clinical prognostic information resources of 478 COAD samples in the TCGA database were included in the study. Two data sets (GSE37364, GSE110224) from the GEO database were selected for verification. Using Kaplan - Meier curves, univariate analysis and multivariate survival analysis to assess the relationship FUNDC1 level of expression and clinicopathological parameters and overall survival. The protein-protein interaction network of FUNDC1 was constructed by String and GeneMANIA databases. Screen the genes co-expressed with FUNDC1 in Oncomine and GEPIA. And use the TIMER database and GEPIA to explore the relationship between FUNDC1 and immune cell infiltration and surface markers.

Results:

FUNDC1 is obviously highly expressed in tumor samples and is related to the poor prognosis of patients. Univariate and multivariate analysis showed that FUNDC1 was related to advanced TNM staging. FUNDC1 mainly interacts with mitochondrial autophagy-related proteins. FUNDC1 is related to immune infiltration and mainly affects CD8+ T cells and macrophages.

Conclusion:

The research has proved that FUNDC1 is closely related to the infiltration of tumor microenvironment immune cells in COAD samples and to the poor prognosis of COAD patients, which may provide new treatment ideas and targets.

Highlights:

1, Analyze the difference in the expression level of COAD samples FUNDC1 in the TCGA database and use GEO data set to verify.

2, R software is used to perform survival analysis, construct timeROC curve, analyze the relationship between FUNDC1 and other clinical characteristics such as age, weight and tumor stage, and perform correlation analysis between FUNDC1 and immune cells in the tumor microenvironment.
3, Multiple databases of TIMER, GEPIA, UALCAN, HPA, String, GeneMANIA and Oncomine were used in the research.

**Introduction:**

Mitochondrial dysfunction is related to the occurrence and development of many diseases, such as metabolic disorders, heart disease, Parkinson disease and cancer[1 – 5]. As a mitochondrial autophagy receptor, FUNDC1 has been reported to mediate the process of mitochondrial autophagy induced under stress conditions (such as hypoxic stimulation)[6, 7]. In metabolic diseases and heart diseases, FUNDC1 is generally considered to have a protective effect because FUNDC1 mediated mitochondrial phagocytosis can reduce the damage caused by intracellular stress (such as hypoxia)[8, 9]. However, the role of FUNDC1 in cancer has not been fully studied. On the one hand, the overexpression of FUNDC1 may promote the progression of certain tumors[10]. On the other hand, it may also inhibit canceration by mediating mitochondrial autophagy[9, 11].

The tumor microenvironment (TME) is a complex integrated system formed by the interaction of tumor cells with surrounding tissues and immune cells[12]. The existence of TME can enhance tumor cell proliferation, migration and immune escape ability, thereby promoting the occurrence and development of tumors[13]. As a part of TME, infiltrating immune cells have been paid more and more attention by researchers. The immune infiltration of TME provides us with clues to understand the progress of cancer diseases, which is of great significance for tumor prognosis or prediction, and can even become a means of cell therapy[14, 15].

Colon carcinoma (COAD) is a common malignant tumor in the gastrointestinal tract[16]. Like other malignant tumors, the cause of this disease is still unclear. The prognosis of COAD is affected by many factors, such as age, location of onset, tumor diameter, pathological classification, whether there is lymph node or distant metastasis, etc. The most crucial predictor should be cancer-centric factors, such as the TNM staging system[17] and molecular markers.

In this article, we used bioinformatics techniques and databases including GEPIA, UALCAN, and Kaplan-Meier Plotter to visualize the expression level of FUNDC1 in COAD and its prognosis. Then, we used the TIMER and GEPIA databases to explore the potential relationship between FUNDC1 expression and the level of immune infiltration. R software was used to process data and perform survival analysis and immune correlation analysis. The results of this study indicate that FUNDC1 may affect the prognosis of patients with COAD through its interaction with immune infiltration.

**Method:**

**Data sources**

The Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) is a cancer research project jointly established
Survival analysis

The raw counts and corresponding clinical information of 455 colon cancer RNA sequencing data were obtained from TCGA. Log rank was used to test Kaplan-Meier (KM) survival analysis to compare the survival difference between FUNDC1 high expression group and low expression group, and time ROC analysis was performed to compare the prediction accuracy and risk score of FUNDC1.

For the KM curve, the p value and the hazard ratio (HR) with 95% confidence interval (CI) are obtained by log rank test and univariate Cox proportional hazard regression. p<0.05 was considered statistically significant. All the above analysis methods and R software packages are implemented using v4.0.3 version R software.

Univariate and multivariate prognostic analysis

In order to further determine the effect of high expression of FUNDC1 on patients with COAD, we used univariate Cox regression analysis to calculate the correlation between the expression level of FUNDC1 and the patient's OS. Then, multivariate analysis was used to assess whether FUNDC1 is an independent prognostic factor for COAD patient survival. When the p value is less than 0.05, Cox regression analysis of FUNDC1 is statistically significant.

Protein-protein interaction analysis

The String database (https://string-db.org/) is a database for searching known and predicted protein interactions[20]. The database can be applied to 2031 species, including 9.6 million proteins and 13.8 million protein interactions. By importing FUNDC1 into String, information about the protein-protein interaction (PPI) can be obtained. GeneMANIA (http://genemania.org/) is a database that can generate hypotheses about gene function, analyze gene lists, and analyze gene priority based on function[21]. Given a list of query genes, it finds genes with similar functions based on rich genomics and proteomics, and weights them based on predicted values.

Screen for co-expressed genes
The Oncomine (https://www.oncomine.org/resource/main.html) is a large tumor gene chip database, covering 65 gene chip data sets, 4700 chips and 480 million gene expression data. It can be used to analyze gene expression differences, find outliers, and predict co-expressed genes[22]. The Oncomine database was used to screen the genes co-expressed with FUNDC1 in COAD samples. The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database integrates current cancer genomics data, making it easier and faster to mine data and perform dynamic analysis of gene expression profile data. The database can be used to verify the relationship between genes.

**Immune Infiltration and Correlation Analysis with Immune**

The Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) database uses high-throughput sequencing (RNA-Seq expression profile) data to analyze the infiltration of immune cells in tumor tissues[23]. Enter FUNDC1 into the database to evaluate the correlation between the expression of FUNDC1 in COAD patients and the abundance of 6 infiltrating immune cells (CD8$^+$ T cells, CD4$^+$ T cells, B cells, dendritic cells, macrophages and neutrophils) and the relationship between FUNDC1 gene expression and tumor purity.

Spearman correlation analysis between FUNDC1 and immune score. The dataset used contains mRNA sequence data from COAD tumors in 455 TCGA databases. A single correlation map is realized by the R software package ggstatsplot, and the multi-gene correlation map is displayed by the R software package pheatmap. We use Spearman's correlation analysis to describe the correlation between quantitative variables that do not have a normal distribution. A p value of less than 0.05 is considered statistically significant. All the above analysis methods are implemented by R v4.0.3.

**Result:**

**Patients clinical information**

The RNA sequencing data and detailed clinical prognostic information resources of 478 COAD samples in the TCGA database were included in the study. All patients were divided into FUNDC1 high expression group 239 cases and low expression group 239 cases. And summarized the patient's age, gender, histological grade, pathological stage (T, N and M), OS time and survival results and other clinical information (Table 1).
Table 1
The corresponding clinical information of patients with different expression levels of FUNDC1 obtained from the TCGA database

| Characteristic          | Low expression of FUNDC1 | High expression of FUNDC1 | p   |
|-------------------------|--------------------------|---------------------------|-----|
| n                       | 239                      | 239                       |     |
| Gender, n (%)           |                          |                           | < 0.001 |
| Female                  | 93 (19.5%)               | 133 (27.8%)               |     |
| Male                    | 146 (30.5%)              | 106 (22.2%)               |     |
| Age, n (%)              |                          |                           | 0.050 |
| <=65                    | 86 (18%)                 | 108 (22.6%)               |     |
| >65                     | 153 (32%)                | 131 (27.4%)               |     |
| OS event, n (%)         |                          |                           | < 0.001 |
| Alive                   | 189 (39.5%)              | 146 (30.5%)               |     |
| Dead                    | 50 (10.5%)               | 93 (19.5%)                |     |
| Pathologic stage, n (%) |                          |                           | 0.023 |
| Stage I                 | 43 (9.2%)                | 38 (8.1%)                 |     |
| Stage II                | 96 (20.6%)               | 81 (16.9%)                |     |
| Stage III               | 69 (14.8%)               | 74 (15.5%)                |     |
| Stage IV                | 29 (6.2%)                | 37 (7.9%)                 |     |
| T stage, n (%)          |                          |                           | 0.446 |
| T1                      | 3 (0.6%)                 | 8 (1.7%)                  |     |
| T2                      | 44 (9.2%)                | 39 (8.2%)                 |     |
| T3                      | 160 (33.5%)              | 163 (34.2%)               |     |
| T4                      | 31 (6.5%)                | 29 (6.1%)                 |     |
| N stage, n (%)          |                          |                           | 0.008 |
| N0                      | 146 (30.5%)              | 128 (26.8%)               |     |
| N1                      | 48 (10%)                 | 60 (12.6%)                |     |
| N2                      | 45 (9.4%)                | 51 (10.6%)                |     |
| M stage, n (%)          |                          |                           | 0.381 |
Table 1
The corresponding clinical information of patients with different expression levels of FUNDC1 obtained from the TCGA database

| Characteristic | Low expression of FUNDC1 | High expression of FUNDC1 | p |
|---------------|--------------------------|---------------------------|---|
| M0            | 177 (42.7%)              | 172 (41.4%)               |   |
| M1            | 29 (7%)                  | 37 (8.9%)                 |   |

Expression of FUNDC1 in tumor tissues

Compared with normal tissues in TCGA, the expression of FUNDC1 was significantly increased in COAD samples (Fig 1B, p=1.1e−13). Two data sets (GSE37364, GSE110224) from the GEO database were selected for verification, which also showed that the expression of FUNDC1 was significantly up-regulated in tumor tissues (Fig 2A, B; p=7.5e-05, 5.9e-05). Searching in the HPA database revealed that the FUNDC1 encoded protein FUN14 domain-containing protein 1 was up-regulated in tumor tissues (Fig 2C, D).

The relationship between FUNDC1 high expression and clinical information of COAD patients

When analyzing the correlation between FUNDC1 expression level and clinicopathological parameters in COAD samples (Fig 1), the results showed that FUNDC1 mRNA level was not significantly different with age (p=0.578), gender (p=0.731), and weight (p=0.324). The expression level of FUNDC1 is positively correlated with tumor stage and N stage (p = 1.324e-03, 3.278e-04).

Survival analysis

The KM curve was constructed according to the method mentioned above, and the results showed that the high expression of FUNDC1 in COAD showed a shorter overall survival (OS, p=8.58e-3, Fig3B). The Time ROC curve shows that FUNDC1 has a certain predictive power for the prognosis of COAD patients (5-Years, AUC=0.76, 95%CI (0.695-0.813), Fig3C). In the univariate Cox model, high FUNDC1 expression and high pathological grade and staging (TNM) are both negative predictors of OS in COAD patients. In the multivariate regression analysis, the expression level of FUNDC1 was shown to be related to OS as an independent factor (Fig4).

PPI network construction

The String database was used to construct the PPI network of FUNDC1, and the top 10 proteins that interact with FUNDC1 and their corresponding gene names, annotations and scores were listed (Fig 5A).
These proteins include: MAP1LC3B, PGAM5, MAP1LC3A, ULK1, ATG5, GABARAPL1, GABARAPL2, GABARAP, ATG12. MAP1LC3B, Microtubule-associated proteins 1A/1B light chain 3B, is ubiquitin-like modifier that involved in formation of autophagosomal vacuoles. Plays a role in mitophagy which contributes to regulate mitochondrial quantity and quality by eliminating the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production[24,25]. Figure 5B shows the proteins predicted to interact with FUNDC1 in the GENEMANIA database and the enrichment pathways of related genes.

Screen for co-expressed genes

In order to further study the mechanism of FUNDC1 in COAD, we used the Oncomine database to mine the genes co-expressed with FUNDC1 in COAD samples. FUNDC1 is co-expressed with 104 genes, of which the highest correlation coefficient is KDM6A. The GEPIA database was selected to further analyze the relationship between FUNDC1 and KDM6A (Fig 6). KDM6A encodes lysine-specific demethylase 6A protein, which mainly plays a role in the regulation of gene expression[26].

Immune Infiltration and Correlation Analysis with Immune

We used the TIMER database to analyze the correlation between FUNDC1 expression and six types of infiltrating immune cells (CD8+ T cells, CD4+ T cells, B cells, dendritic cells, macrophages and neutrophils) and tumor purity (Fig 7). The results showed that the level of FUNDC1 was significantly correlated with CD8+ T cells (r=0.191, p=1.04e-04) and macrophages (r=0.155, p=1.82e-03). There was no significant correlation with tumor purity (r=0.007, p=8.38e-01), CD4+ T cells (r=0.011, p=8.29e-01), neutrophils (r=0.028, p=5.71e-01), and dendritic cells (r=0.012, p=8.03e-01). P <0.05 is considered statistically significant. The results of immune correlation analysis using R software showed that FUNDC1 levels were correlated with B cells (p=0.012, Spearman=-0.12), CD4+ T cells (p=0.028, Spearman=-0.10), endothelial cell (p=9.7e-05, Spearman=-0.18), and NK cells (p=0.009, Spearman=-0.12), CD8+ T cells (p=1.9e-04, Spearman=-0.17), macrophages (p=2.36e-05, Spearman=-0.20) are negatively correlated. Among them, it has the strongest correlation with macrophages. P <0.05 is considered statistically significant (Fig 7).

Discussion:

In this article, we used TCGA data from TIMER and GEPIA to explore the expression level of FUNDC1 in COAD samples, and chose two datasets (GSE37364, GSE110224) from GEO to verify. The results showed that FUNDC1 was significantly up-regulated in COAD. The protein-level verification in the HPA database further confirmed that FUNDC1 is highly expressed in COAD samples. The ggrisk, survival and Survminer packages of R software were used to process the RNA sequencing data of 455 COAD tumor samples in TCGA and draw the KM curve. The results showed that the high expression of FUNDC1 was related to the poor prognosis of COAD patients (p = 8.58e-3). TimeROC analysis was performed through
the timeROC package to compare the prediction accuracy and risk score of the FUNDC1 gene (5 – Years, AUC = 0.76). It indicated that the expression level of FUNDC1 has a certain predictive significance for the prognosis of COAD patients. Through univariate Cox and multivariate Cox analysis, and to explore the relationship between the expression level of FUNDC1 in COAD samples in the TCGA database and the corresponding clinical characteristics such as pathological staging and OS, the results showed that the high expression of FUNDC1 is closely related to high pathological grade and staging (TNM). And FUNDC1 is an independent predictor of COAD.

Mitochondria perform a variety of functions in cells: produce adenosine triphosphate to provide energy for cells; buffer calcium ions and maintain calcium homeostasis; produce and regulate reactive oxygen species (ROS); Through the mitochondrial membrane permeability transition pore to regulate cell apoptosis[27]. Mitophagy is an important mitochondrial quality control mechanism, which is used to selectively target and remove damaged or dysfunctional mitochondria[28]. Mitophagy has two sides. On the one hand, mitochondrial autophagy within the normal range can help maintain the stability of the cell environment, and on the other hand, too high or too low mitochondrial autophagy can cause diseases. Recent studies have shown that mitochondrial autophagy is closely related to many human diseases, including cardiovascular diseases, tumors, inflammation and neurodegenerative diseases[1, 3]. And FUNDC1 as a new receptor protein that mediates mitochondrial autophagy, in-depth understanding of its potential role in related diseases can open up new ways for disease prevention and treatment. Previous studies have found that PINK1, Parkin, NIX/BNIP3 and BCL2L13 are important factors in the regulation of mitochondrial autophagy[29–31]. In addition, mitochondrial dynamic regulators MFN1, MFN2, OPA1, DRP1, FIS1, mitochondrial matrix proteases LONP and CLPP, mitochondrial inner membrane proteases YME1L1, AFG3L2 and SPG7 all play vital functions in mitochondrial quality control[32–35]. FUNDC1, encodes FUN14 domain-containing protein 1, as an activator of hypoxia-induced mitochondria, participates in the significant process of mitochondrial quality control. FUNDC1 is a mitochondrial membrane protein that mediates mitochondrial autophagy in mammalian cells and consists of 155 amino acids. FUNDC1 is located on the mitochondria and contains three transmembrane domains, as well as the N-terminal domain exposed to the cytoplasm and the C-terminal domain inserted into the outer mitochondrial membrane[6]. The N-terminal exposed to the cytoplasm contains a typical LC3 interaction region (LIR). Under physiological conditions, FUNDC1 stably exists in the mitochondrial membrane in the form of phosphorylation without inducing the occurrence of mitochondrial autophagy. However, when hypoxia or mitochondrial uncoupling, unc-51 like kinase 1 (ULK1) phosphorylates Ser17 on FUNDC1, and phosphoglycerate mutase family member 5 (PGAM5) makes Ser13 dephosphorylation promotes the interaction between FUNDC1 and LC3 and the occurrence of mitochondrial autophagy[6]. Wu et al.[36] through ULK1 binding defective mutants and FUNDC1 knock-out experiments confirmed that FUNDC1 inactivation can disrupt the translocation of ULK1 to damaged mitochondria and inhibit mitochondrial autophagy. They proposed that FUNDC1 is the mitochondrial localization substrate of ULK1 and may have ULK1 adaptor effect.

There have been some studies on the role of FUNDC1 in tumorigenesis and development, but the specific mechanism is still not very clear. The expression of FUNDC1 in cervical cancer cells is significantly higher
than that in adjacent tissues, and the high expression of FUNDC1 is negatively correlated with the prognosis of cervical cancer patients, and can be used as an independent prognostic factor for overall survival and disease-free survival[37]. Silencing FUNDC1 expression by short hairpin ribonucleic acid can significantly inhibit tumor cell proliferation, induce tumor cell apoptosis, and enhance the sensitivity of cells to cisplatin and ionizing radiation. It is suggested that FUNDC1 can be used as a biomarker for the prognosis of patients with cervical cancer, and it may become a new therapeutic target to improve the anti-tumor effect of radiotherapy and chemotherapy. Regarding breast cancer[38], in vitro experiments on FUNDC1 function gain and loss have shown that FUNDC1 significantly stimulates breast cancer cell proliferation, metastasis and invasion. Further increasing the level of FUNDC1 can promote the nuclear translocation and activity of nuclear factor of activated T cells 1 (NFATc1). Nuclear NFATc1 binds to the BMI1 oncogene promoter and transcriptionally up-regulates its expression. Overexpression of BMI1 can rescue the loss of FUNDC1 function. The prognosis of breast cancer patients with co-expression of FUNDC1 and BMI1 in vivo is worse than that of patients without either expression. It shows that FUNDC1 may activate the Ca\(^{2+}\)-NFAT-BMI1 axis to promote breast cancer progression, and this approach is expected to become a new target for breast cancer treatment[38]. But on the other hand, some studies have shown that FUNDC1 has an inhibitory effect on tumors. FUNDC1 mediated mitochondrial phagocytosis can inhibit the occurrence of liver cancer. The specific knockout of FUNDC1 in liver cells promotes the initiation and progression of HCC induced by the chemical carcinogen diethylnitrosamine. The study also found that FUNDC1 transgenic hepatocytes have a protective effect on the development of HCC[11].

Grasping the relationship between FUNDC1 and immune cell infiltration in TME may help to understand the mechanism of tumorigenesis and development. In this article, we found that the expression of FUNDC1 in COAD samples has a certain correlation with immune cell infiltration. The results showed that FUNDC1 was significantly related to CD8\(^{+}\) T cells and macrophages, but not to tumor purity. TME is a complex integrated system formed by the interaction of tumor cells with surrounding tissues and immune cells[12]. It is generally believed that genes that are highly expressed in TME cells are negatively correlated with tumor purity. Correspondingly, genes that are highly expressed in tumor cells are expected to be positively correlated with tumor purity[39]. The expression level of FUNDC1 in COAD samples has nothing to do with tumor purity, indicating that there is no significant difference between its expression in tumor cells and TME. CD8\(^{+}\) T cells, also known as cytotoxic T cells (CTL), are recognized as the main anti-tumor immune effector cells[40]. The MHC I on the surface of CD8\(^{+}\) T cells can specifically identify tumor-associated antigens, and when combined with tumor cells, it produces perforin and other cytotoxins, which kill cancer cells but does not affect normal cells. It is often used as the target of targeted drugs. However, it has been clinically observed that CD8 \(^{+}\) T cells that can identify epitopes in the tumor in situ or in peripheral blood are often eliminated in the tumor microenvironment[41]. It has been proved that these CD8 \(^{+}\) T cell membranes are combined with FasL or PDL-1 or TGF-\(\beta\) secreted by tumor cells to promote the apoptosis of anti-tumor effect CD8 \(^{+}\) T cells in TIL; or it can lead to the loss of tumor antigens and the down-regulation of MHC I molecules, which in turn allows the tumor to escape the host immune system[42]. This affects all T cell responses to prevent over-activation and tissue damage. For
example, PD-L1 expressed by tumor cells can reduce T cell proliferation, survival and production of cytokines by activating the PD-1/PD-L1 signaling pathway. In addition, the relationship between FUNDC1 expression level and immune cell markers is not always consistent, such as STAT3, IL-21R, IL12RB2, STAT1 (Table 2). This indicates that there may be specific interactions between FUNDC1 and different subtypes of immune cells. We also found a strong correlation between FUNDC1 and regulatory T cells (Treg) cell markers. Treg account for only 5% of the CD4+ helper T cell subset, but they play an important role in regulating the in situ immune response[43]. It can be found in different tumors that tumor cells can recruit Treg into the tumor microenvironment, so the existence and function of Treg seem to be inversely proportional to the prognosis of cancer. Human Treg lacks a clear phenotypic classification. Natural Treg (nTreg; CD4+, CD25, highFOXP3+) can control cancer-related inflammation; induced Treg (iTreg) seems to down-regulate the tumor response in situ and promote cancer cell growth.
Table 2
the relationship between FUNDC1 and different cell markers of immune cells

| Cell type       | Gene marker | None  |        |        |        |        | Purity |        |        |        |        |
|-----------------|-------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                 |             | Cor   |        | P      | Cor    |        | P      |        | Cor    |        | P      |
| B cell          | CD19        | -0.095| *      | 0.095  | -0.108 | *      | 0.108  | -0.071 | 0.24   | -0.076 | 0.64   |
|                 | CD20        | 0.124 | **     | 0.124  | 0.13   | **     | 0.13   | -0.025 | 0.67   | -0.22  | 0.17   |
|                 | CD38        | -0.058| 0.218  | -0.058 | -0.077 | 0.125  | -0.077 | -0.092 | 0.13   | -0.036 | 0.82   |
| CD8⁺Tcell       | CD8A        | -0.038| 0.418  | -0.038 | -0.042 | 0.401  | -0.042 | -0.11  | *      | 0.016  | 0.92   |
|                 | CD8B        | 0.063 | 0.181  | 0.163  | 0.191  | **     | 0.191  | 0.092  | 0.13   | 0.02   | 0.9    |
| Tfh             | BCL6        | -0.05 | 0.283  | -0.05  | -0.024 | 0.633  | -0.024 | -0.17  | **     | 0.24   | 0.13   |
|                 | ICOS        | 0.108 | *      | 0.108  | 0.012  | 0.809  | 0.012  | 0.11   | 0.071  | 0.13   | 0.41   |
|                 | CXCR5       | -0.087| 0.064  | -0.087 | -0.084 | 0.092  | -0.084 | -0.061 | 0.31   | -0.14  | 0.37   |
| Th1             | T-bet       | -0.115| **     | 0.115  | -0.105 | *      | 0.105  | -0.14  | *      | 0.029  | 0.86   |
|                 | STAT4       | -0.039| 0.405  | -0.039 | -0.04  | 0.417  | 0.04   | -0.18  | **     | 0.18   | 0.27   |
|                 | IL12RB2     | -0.092| *      | 0.092  | -0.097 | *      | 0.097  | -0.012 | 0.84   | 0.20   | 0.20   |
|                 | WSX1        | 0.019 | 0.678  | 0.019  | 0.024  | 0.628  | 0.024  | 0.628  | 0.10   | 0.096  | 0.12   |
|                 | STAT1       | 0.092 | *      | 0.092  | 0.109  | *      | 0.109  | -0.057 | 0.35   | 0.14   | 0.39   |
|                 | IFN-γ       | -0.021| 0.656  | -0.021 | -0.001 | 0.991  | 0.001  | -0.066 | 0.27   | 0.17   | 0.28   |
|                 | TNF-α       | -0.066| 0.162  | -0.066 | -0.058 | 0.244  | 0.058  | -0.10  | 0.091  | 0.11   | 0.47   |
| Th2             | GATA3       | -0.059| 0.209  | -0.059 | -0.078 | 0.116  | 0.078  | -0.083 | 0.17   | 0.072  | 0.66   |
|                 | CCR3        | -0.058| 0.213  | -0.058 | -0.096 | 0.096  | 0.096  | -0.16  | **     | -0.10  | 0.52   |
|                 | STAT6       | -0.036| *      | 0.036  | -0.042 | 0.116  | 0.042  | -0.11  | 0.078  | -0.055 | 0.73   |
|                 | STAT5A      | -0.102| *      | 0.102  | -0.105 | *      | 0.105  | -0.11  | 0.069  | 0.059  | 0.71   |
| Th9             | TGFBR2      | 0.277 | ***    | 0.277  | 0.267  | ***    | 0.267  | 0.029  | 0.63   | 0.19   | 0.23   |
|                 | IRF4        | -0.101| **     | 0.101  | -0.116 | **     | 0.116  | -0.13  | *      | -0.12  | 0.47   |
|                 | PU1         | -0.173| ***    | 0.173  | -0.19  | ***    | 0.19   | -0.15  | **     | 0.24   | 0.14   |
| Th17            | STAT3       | 0.09  | *      | 0.09   | 0.112  | *      | 0.112  | -0.12  | *      | 0.025  | 0.88   |
|                 | IL-21R      | -0.165| ***    | 0.165  | -0.179 | ***    | 0.179  | -0.14  | *      | -0.069 | 0.67   |
|                 | IL-23R      | 0.145 | ***    | 0.145  | 0.157  | ***    | 0.157  | 0.084  | 0.17   | -0.10  | 0.53   |
| Cell type       | Gene marker | None  | Purity | Tumor | Normal |
|-----------------|-------------|-------|--------|-------|--------|
| IL-17A          | 0.004       | 0.934 | 0.013  | 0.788 | 0.026  | 0.67   | -0.042 | 0.80 |
| Th22 CCR10      | -0.306 ***  | -0.314 *** | -0.13 * | -0.29 | 0.064  |
|                 | AHR         | 0.151 *** | 0.177 *** | -0.13 * | 0.36   | *      |
| Treg FOXP3      | -0.092 **   | -0.1 ** | -0.084 | 0.16  | 0.076  | 0.64   |
|                 | CD25        | -0.131 ** | -0.131 ** | -0.15 ** | 0.096  | 0.55   |
|                 | CCR8        | 0.025  | 0.597  | 0.038  | 0.448  | -0.08  | 0.19   | 0.093  | 0.56 |
| T-cell exhaustion | PD-1        | -0.162 *** | -0.163 *** | -0.11 | 0.077  | -0.064 | 0.69   |
|                 | CTLA4       | -0.065  | 0.166  | -0.048 | 0.338  | -0.09  | 0.14   | 0.16   | 0.32 |
|                 | LAG3        | -0.159 ** | -0.148 *** | -0.026 | 0.67   | -0.054 | 0.74   |
|                 | TIM-3       | -0.049  | 0.294  | -0.036 | 0.464  | -0.14  | *      | 0.45   | *** |
| Macrophage      | CD68        | -0.161 *** | -0.156 *** | -0.13 * | 0.11   | 0.49   |
|                 | CD11b       | -0.157 *** | -0.16 *** | -0.13 * | 0.43   | ***    |
| M1 INOS         | -0.219 ***  | -0.197 *** | -0.15 ** | 0.2    | 0.22   |
|                 | IRF5        | 0.129 ** | 0.12   | 0.13   | -0.0052 | 0.97   |
|                 | COX2        | -0.067  | 0.149  | -0.062 | 0.212  | -0.096 | 0.11   | 0.27   | *    |
| M2 ARG1         | 0.014       | 0.771  | 0.019  | 0.709  | -0.061 | 0.31   | 0.032  | 0.84   |
|                 | MRC1        | -0.144 *** | -0.147 *** | -0.12 * | 0.33   | *      |
|                 | MS4A4A      | -0.061  | 0.193  | -0.058 | 0.242  | -0.12  | *      | 0.44   | *** |
| TAM CCL2        | 0.072       | 0.123  | 0.062  | 0.213  | -0.091 | 0.13   | 0.32   | *      |
|                 | CD80        | -0.058  | 0.219  | -0.055 | 0.270  | -0.12  | *      | 0.25   | 0.12 |
|                 | CD86        | -0.032  | 0.492  | -0.015 | 0.765  | -0.15  | **     | 0.5    | *** |
|                 | CCR5        | -0.012  | 0.798  | -0.003 | 0.944  | -0.11  | 0.059  | 0.081  | 0.62 |
| Monocyte        | CD14        | -0.222 *** | -0.235 *** | -0.18 ** | 0.36   | *      |
|                 | CD16        | -0.198 *** | -0.203 *** | -0.11  | 0.068  | 0.21   | 0.19   |
|                 | CD115       | -0.142 *** | -0.16 *** | -0.14 ** | 0.24   | 0.13   |
| Neutrophil      | CD66b       | -0.137 *** | -0.148 *** | 0.051  | 0.40   | -0.12  | 0.47   |
|                 | CD15        | 0.117 ** | 0.141 *** | 0.048  | 0.42   | 0.085  | 0.6    |
|                 | CD11b       | -0.157 *** | -0.16 *** | -0.13 * | 0.43   | **     |
| Cell type   | Gene marker | None | Purity | Tumor | Normal |
|------------|-------------|------|--------|-------|--------|
| NK cell    | XCL1        | -0.048 | 0.308  | 0.039 | 0.328  |
| CD7        | -0.213      | ***   | -0.22  | ***   | -0.13  |
| KIR3DL1    | -0.155      | ***   | -0.145 | ***   | -0.087 |
| Dendritic cell | CD1C   | 0.055 | 0.244  | 0.049 | 0.433  |
| CD141      | -0.017      | 0.715 | 0.018  | 0.718 | 0.095  |
| CD11c      | -0.157      | ***   | -0.151 | ***   | -0.18  |

(None, Correlation without adjustment. Purity, Correlation adjusted by tumor purity. Cor, R value of Spearman's correlation. *P < 0.05; **P < 0.01; ***P < 0.001)

In this article, we explored the relationship between FUNDC1 and COAD immune infiltration and prognosis, and the results showed that the high expression of FUNDC1 is related to the poor prognosis of COAD. The main deficiencies of this study are the lack of verification of the mechanism and pathways of FUNDC1 at the cellular and molecular levels. Secondly, the post-translational modifications of FUNDC1 such as phosphorylation and ubiquitination may interfere with its function, which is the direction we will study next.

**Conclusion:**

We use bioinformatics technology to study the possible mechanism of FUNDC1 and its interacting genes and proteins in the occurrence and development of COAD. The results prove that FUNDC1 is closely related to the infiltration of TME immune cells in COAD samples, which may provide new treatment ideas and targets.

**Abbreviations**

TME: tumor microenvironment; COAD: Colon carcinoma; HR: hazard ratio; CI: confidence interval; PPI: protein-protein interaction; OS: overall survival; ROS: reactive oxygen species; ULK1: unc-51 like kinase 1; PGAM5: phosphoglycerate mutase family member 5; CTL: cytotoxic T cells; Treg: regulatory T cells.

**Declarations**

**Acknowledgements**

Not applicable.
Funding

Not applicable.

Ethics approval and consent to participate

The data used in this article are all from online public databases, so ethical and moral issues are not involved.

Authors' contributions

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Consent for publication

Written consent was obtained from the patient for publication of this study and accompanying images.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 1

FUNDC1 expression level. A: the differential expression of FUNDC1 in tumor tissues and adjacent tissues; B: FUNDC1 is highly expressed in COAD samples ($p=1.1\times10^{-13}$); C-G: the expression level of FUNDC1 was not significantly different with age ($p=0.578$), gender ($p=0.731$), and weight ($p=0.324$), but positively correlated with tumor stage and N stage ($p = 1.324\times10^{-3}, 3.278\times10^{-4}$).
Figure 2

GEO and HPA databases were used to verify differences in FUNDC1 expression levels. A: GSE37364 data set verifies that FUNDC1 is highly expressed in COAD samples (p=7.5e-05); B: GSE110224 data set verifies that FUNDC1 is highly expressed in COAD samples (p=5.9e-05); C: In the HPA database, it can be seen that FUN14 domain-containing protein 1 is highly expressed in COAD tissues (10X).
Figure 3

The prognostic analysis of FUNDC1 for COAD patients. A: gene expression and survival time and survival status in the TCGA data set, where the top represents the scatter plot of the gene expression from low to high; the middle represents the scatter plot distribution of survival time and survival status corresponding to the gene expression of different samples; the figure below represents the expression heat map of the gene; B: The KM survival curve distribution of FUNDC1 in the TCGA data set, where different groups are tested by log rank, HR (Low exp) represents the risk coefficient of the low expression group relative to the
sample of the high expression group, and FUNDC1 is a risk factor (HR=0.589); C: Time-dependent ROC analysis of FUNDC1.

### Table A

| Characteristic       | Total (N) | HR (95% CI) | p Value |
|----------------------|-----------|-------------|---------|
| Age (>60 vs. <60)    | 478       | 1.783 (1.324–2.407) | 0.132   |
| Gender (Male vs. Female) | 478      | 1.030 (0.673–1.384)  | 0.648   |
| T stage (T3&T4 vs. T1&T2) | 478    | 3.134 (2.176–3.974)  | <0.001  |
| N stage (N1 vs. N0)  | 349       | 3.548 (2.432–6.319)  | <0.001  |
| M stage (M1 vs. M0)  | 478       | 4.098 (3.107–5.643)  | <0.001  |
| Histologic grade (G3&G4 vs. G1&G2) | 470  | 2.679 (2.042–3.917)  | <0.001  |
| FUNDC1 (High vs. Low) | 478       | 3.422 (2.767–4.341)  | <0.001  |

### Table B

| Characteristic       | Total (N) | HR (95% CI) | p Value |
|----------------------|-----------|-------------|---------|
| Age (>60 vs. <60)    | 478       | 1.783 (1.324–2.407) | 0.186   |
| Gender (Male vs. Female) | 478      | 1.030 (0.673–1.384)  | 0.648   |
| T stage (T3&T4 vs. T1&T2) | 478    | 2.674 (1.528–2.974)  | 0.032   |
| N stage (N1 vs. N0)  | 349       | 2.087 (1.091–4.779)  | <0.001  |
| M stage (M1 vs. M0)  | 478       | 2.374 (1.263–3.951)  | 0.217   |
| Histologic grade (G3&G4 vs. G1&G2) | 470  | 1.485 (1.029–2.549)  | 0.143   |
| FUNDC1 (High vs. Low) | 478       | 3.377 (2.583–4.634)  | <0.001  |

### Figure 4

Univariate and multivariate analysis of the relationship between FUNDC1 expression level and other clinicopathological parameters and OS.

### Figure 5

PPI network of FUN14 domain-containing protein 1. A: the data comes from the String database, which lists ten interacting proteins and corresponding gene names; B: the data comes from the GeneMANIA database to screen genes with similar functions to FUNDC1.
Figure 6

Genes co-expressed with FUNDC1 in COAD samples. A: screen the co-expressed genes of FUNDC1 in COAD samples in the Oncomine database; B: perform correlation analysis between FUNDC1 and KDM6A in the GEPIA database ($r=0.460; p<0.001$); C&D: survival analysis of KDM6A.
Figure 7

FUNDC1 expression and immune cell infiltration and immune correlation analysis. A: the level of FUNDC1 was significantly correlated with CD8+ T cells and macrophages; B: FUNDC1 levels were correlated with B cells (p=0.012, Spearman=-0.12), CD4+ T cells, endothelial cell, NK cells, CD8+ T cells and macrophages are negatively correlated.