STING promotes proliferation and induces drug resistance in colorectal cancer by regulating the AMPK mTOR pathway

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Research Article
STING promotes proliferation and induces drug resistance in colorectal cancer by regulating the AMPK-mTOR pathway

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Abstract Despite a rapidly growing body of pertinent literature, the relationship between stimulator of interferon genes (STING) protein expression and the progression of colorectal cancer (CRC) remains controversial. This study aimed to investigate the potential roles of STING as a prognostic biomarker and therapeutic
target in the medical management of CRC. STING expression was examined by immunohistochemistry to evaluate its association with clinicopathological factors. Moreover, the effects of STING on CRC cell proliferation, migration, invasiveness, and drug resistance were examined. Gene set enrichment analysis was applied to explore potential downstream mechanisms of STING in CRC. Meanwhile, we evaluated the effect of STING on glucose uptake. Our study confirmed that STING expression was significantly up-regulated in CRC tissues, and was associated with TNM stage and a poor prognosis. Additionally, STING promoted cell proliferation, migration, invasiveness, and drug resistance by mediating AMPK-mTOR signaling. Finally, we confirmed that STING regulates energy metabolism in CRC cells. STING may represent a promising prognostic biomarker and therapeutic target for chemosensitization and inhibition of CRC progression.

**Keywords:** Colorectal cancer; STING; Clinicopathological features; Proliferation; Drug resistance

**Introduction**

Colorectal cancer (CRC) ranks third and second respectively in cancer incidence rate and mortality rate. As the latest updated GLOBOCAN 2020 estimate, colorectal cancer, including anal cancer, is estimated to have 1.9 million new cases and it is worth noting that 935000 deaths in 2020 [1]. Although surgery combined with chemotherapy can substantially ameliorate the prognosis, many patients experience poor clinical outcomes due to advanced tumor stage during diagnosis, distant metastasis, and drug resistance. Exploring the in-depth mechanism of colorectal
cancer progression will help to find new biomarkers and provide information for improving treatment.

Stimulator of interferon genes (STING), referred to TMEM173 and STING1, is a key innate immune sensor [2-4]. In higher eukaryotes, the cyclic guanosine monophosphate-adenosine 5’-monophosphate (AMP) synthase (cGAS)-STING pathway activation has been characterized as an inflammatory mechanism that is induced by cytosolic double-stranded DNA (dsDNA) [5]. Recently, study suggests the potential roles of STING as an independent prognostic biomarker and potential target to improve anticancer immunity in CRC [6]. Although the STING pathway in CRC has not been elucidated fully, multiple studies suggest that it mediates carcinogenesis [7,8]. The rapid proliferation of cancer cells imposes a high energy demand. The pathway of AMP-activated protein kinase (AMPK)-mammalian target of the rapamycin (mTOR) occupied an vital part in the modification of energy metabolism. The AMPK-mTOR pathway is also bound up with tumor drug resistance [9,10]. STING which is related to the regulation of AMPK-mTOR pathway, exists in multiple malignant tumors, such as melanoma, gastric cancer and hepatocellular carcinoma [10-12].

Our previous study demonstrated that fatty acid 2-hydroxylase depletion decreased the chemosensitivity of gastric cancer cells, partially due to the restraint of AMPK pathway [13]. GLI1 overexpression, in combination with AKT-mTOR signaling, induces drug resistance in gastric cancer [14]. However, whether STING mediates tumor regulation through the AMPK-mTOR pathway or can act as a
promising therapeutic target in CRC requires further exploration. Our findings may facilitate the assessment of STING as a diagnostic biomarker and characterize the pathway by which STING regulates CRC.

Materials and methods

Patient specimens

From 2016 to 2018, 32 couples of colorectal cancer and adjacent tissue were obtained from patients undergoing radical surgery in the First Affiliated Hospital of Soochow University. Tissue specimens were stored in a liquid nitrogen tank or formalin tissue fixative immediately after resection. Clinicopathological features can be obtained from the electronic medical record. All procedures involving human participants in this study comply with the Helsinki Declaration. Our study has been approved by the biomedical research ethics committee of the First Affiliated Hospital of Suzhou University (2021-no: 213). We also obtained the written informed consent from the patient or his family. Table 1 lists all clinicopathological features of colorectal cancer samples.

Cell cultures and transfection

CRC cell lines were obtained from the Cell Bank of Shanghai (Shanghai, China). After resuscitation, all cell lines were subcultured for less than 4 months and cultured in RPMI 1640 medium or DMEM (thermo fisher scientific, Carlsbad, California, USA), which contained 10% FBS (GIBCO), with temperature of 37 °C and humidity of 5% CO2.
For transfection. PcDNA3.1-Flag-vector and pcDNA3.1-Flag-STING plasmids encoding human wild-type (WT) STING were obtained from the Public Protein/Plasmid Library (Nanjing: China). The plasmid sequences were verified via Sanger sequencing. After incubation 24 hours, the plasmid (50 nm) was transfected into LoVo cells by Lipofectamine 2000 (Thermo Fisher Scientific) according with the manufacturer's protocol. Subsequent operations were carried out 24-48 h after plasmids transfection.

**Western blot**

RIPA lysis buffer (Sigma Aldrich) was used to lyse cells for 30 minutes to extract total protein [14]. SDS-PAGE was used to isolate the total protein and then transfer the protein to PVDF membrane. These bands were blocked with 5% skimmed milk before incubating with polyclonal antibody (Cell Signaling Technology) overnight. After that, it was incubated with secondary antibody, and finally imaged by chemiluminescence. The results were analysed using ImageJ software (version: 1.4.3, RRID: SCR_003070).

**Immunohistochemistry (IHC) and immunofluorescence staining**

Paraffin embedded tissue section 5 μm. The slices need to be dewaxed, rehydrated, and blocked with 30% hydrogen peroxide. Then dye with hematoxylin and rinse with tap water. Next, 0.5% hydrochloric acid ethanol solution was added; the sample was soaked for several seconds and then rinsed with tap water. Subsequently, the sample was stained with eosin solution. The samples were dehydrated with ethanol and xylene. The tissues were sealed with neutral resin. In addition, the sections were incubated with antibodies (Cell Signaling Technology, USA; 1:200 dilution) after blocked, then rinsed and incubated with secondary antibodies for 30 minutes. As
mentioned earlier, the results was depended on the color intensity and positive cells [13]. The proportion fraction of positive cells in immunohistochemical sections (0, < 5%; 1, 5-25%; 2, 25-50%; 3, 50-75%; 4, > 75%) multiplied by dye intensity fraction; The degree of staining is divided into negative (0-1), weak positive (2-3), positive (4-7) and strong positive (8-12). In this study, the immune response score of 0-4 was negative and 5-12 was positive.

**Bioinformatics analysis**

GSE100179 is based on the platform GPL17586 ([HTA-2_0] Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version], and contains 20 pairs of non-cancerous and colorectal cancer tissues. The gene expression data of 40 samples were used for in-depth analysis. And we used “TCGA” of UALCAN (http://ualcan.path.uab.edu/). The correlation between STING mRNA expression in COAD and individual tumor stage and lymph node metastasis was analyzed. GO and KEGG are indispensable tools for gene annotation. In this study, we analyzed these genes interacting with STING by metascape. At the same time, we also used the online database to retrieve the search tool of interacting genes (STRING; https://string-db.org/). As for exploring co-expression of "STING" and "mTOR", the "TCGA, pancancer Atlas " dataset was chose. CeRNA network is consisted of coding RNA and non-coding RNA. First, we used TargetScan and ENCORI to predicted all STING miRNAs successfully. We then searched miRNAs in COAD on ENCORI. In addition, we used LncBase v.2 to predict the associated lncRNA (PR score > 0.8). Finally, the CeRNA network was successfully constructed via using Cytoscape
software. TIMER (https://cistrome.shinyapps.io/timer/), the immune infiltration abundance was calculated via various immune deconvolution methods. We retrieved “STING; COAD” in “Gene module” and performed immunooosmotic analysis. We also analyzed the expression data of STING in many tumors. All gene expression data can be obtained from UCSC Xena. It is worth mentioning that R-package "ggpubr" can effectively help us obtain all useful charts.

6-Carboxy-fluoresce in phosphoramidate (FAM)-labeled siRNA (siRNA-FAM) uptake

Cells were cultured in 96-well plates for 24 h. Transfection reagent (RNAiMAX/liposome ™ 2000) was added to the medium with 5, 10 or 20 nm FAM-labeled negative control siRNA (FAM siRNA). Then, before adding Lipofectamine RNAiMAX reagent, use 100 μL Opti-MEM (1x), glutamine and 5% fetal bovine serum replaced the medium. Reagents and different concentrations of FAM siRNA were diluted in serum-free Opti-MEM at a ratio of 1:1. For 5 and 24 h incubation, cells were washed with PBS once. Combine the nucleus with 1 μg/L Hoechst 33342 fluorescent DNA probe (Cell Signaling Technology) was incubated for 15 minutes. Using ImageJ software to analyze the image by background subtraction.

STING silencing

IBSBIO synthesized siRNA targeting human STING (specific target sequence: 5’-GCAUCAAGGAAUCGGGUU-3’). An interfering siRNA (5’-UUCUCCGAACGUGACUAGTT-3’) was used as a negative control. Before the experiment, the cells were inoculated in 6-well dishes with 2.5-3 × 105 cells / well and cultured in their respective medium for 24 hours. CRC cells were transfected
using Lipofectamine RNAmax (Thermo Fisher Scientific) reagent, as described previously [13].

**Cell viability**

Cell counting kit-8 (Donjindo, Japan) was adopted to measure cell viability. The cells were cultured in 96 well dishes. After a 24 h incubation, the supernatant from each well was removed. CCK-8 solution and cell culture medium was added according with the manufacturer's protocol. Finally, the absorbance was detected for five consecutive days using a multifunctional microplate reader. Each operation is carried out in triplicate.

**Transwell invasion and migration assay**

Transwell plates (Corning Incorporated, USA) were used for the subsequent experiments. The matrix gel used for the invasion test was slowly thawed on ice at 4°C. The chamber insert used for invasion analysis was coated with a dilute matrix gel and dried at 37 °C. Cells (1 × 10^5) placed in the upper chamber of each incubator, which were suspended in serum-free medium; The inferior chamber contained 20% FBS intact medium to induce the cells to move downward. After 24 hours of incubation, the cells were fixed and stained. Finally, the transitional cells were quantified by inverted microscope (Nikon).

**Glucose uptake assay**

After siRNA transfections, cells were treated with 0.1 mM 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1, 3-diazol-4-yl) Amino)-2-Deoxyglucose) (Invitrogen) in culture medium. Incubating the plates at 37 °C and 5% CO2 for a period of time, as described
above. We used the unified acquisition settings on the fluorescence microscope (Leica) to obtain images. The average fluorescence intensity was analyzed by image J.

**Statistical analysis**

The mean ± SD represents all data, and GraphPad Prism 8.0 (GraphPad Software, Inc.) is applied for statistical analysis. The $\chi^2$ or Fisher's exact test was adopted to evaluate relationship between categorical clinicopathological variables and STING expression levels. Comparisons among two experimental groups were performed using two-tailed Student's t-test. For comparison between groups, Two-way ANOVA and Sidak's post-hoc test were often used. $P < 0.05$ represents a statistically significant difference.

**Results**

**In CRC, STING expression is up-regulated and correlated with advanced tumor stage and poor prognosis**

Analysis of the COAD GEO dataset (GSE100179) and TCGA database, suggested that the expression level of STING was increased significantly in CRC tissues (Fig. 1a,1b). And, the outcomes of UALCAN found that the mRNA of STING was particularly high in patients with higher histological grade (poor differentiation) and higher clinical stage (Fig. 1c, 1d). IHC staining showed STING expression in 17 of 32 tumor samples and 4 of 32 paired adjacent tissues (Fig. 1e-1g). Furthermore, the expression level of STING was bound up with whether patients had lymph node metastasis. The STING expression of CRC patients with lymph node metastasis was significantly increased
(Fig. 1h, \(P <0.01\)). Both Western blot and IHC staining results demonstrated that the expression of STING in CRC was increased matched with surrounding normal tissues (Fig. 1i-1j).

The expression level of STING is related to the clinicopathological indexes of patients with CRC

STING expression levels were classified according to IHC staining scores, and related to clinicopathological indicators. STING expression was associated with lymphatic metastasis \( (P <0.05) \) and TNM stage \( (P <0.05) \). However, no significant associations were found among the following parameters: age, sex, tumor diameter, depth of tumor invasion, and tumor differentiation (Table 1). The \( P \)-values in each group were \( >0.05 \) and there was no statistical difference between groups; however, statistical power may be limited by the number of samples and the effect of the cut-off value of STING expression.

Effects of targeting STING expression on proliferation, migration, invasion, and drug sensitivity of CRC cell lines

Western blotting detected STING protein expression in all five CRC cell lines. Expression levels were elevated in all CRC cell lines except LoVo; furthermore, expression levels in the HCT116 and SW480 lines were similar (Fig. 2a). Transfection of HCT116 and SW480 cells with STING-siRNA (Fig. 2b) significantly reduced STING expression (Fig. 2c). The number of migrating and invasive cells in the STING-knockdown (STING-KD) group was significantly lower than that in control group (Fig. 2d-2e, \( P < 0.05 \)). The results show that different expression levels of STING can
influence the migration and invasion of CRC cells. CCK-8 analysis confirmed that the cell proliferation in STING-KD group decreased significantly at 72 hours compared with the untreated group and the negative control group (NC-siRNA) (Fig. 2f, P < 0.05). The above results suggested that elevated STING expression may promote CRC cell proliferation. Furthermore, The CCK-8 assay also demonstrated that the CRC cells in STING-siRNA group were highly sensitive to 72 h of 5-FU exposure (Fig. 2g, P <0.05).

Effects of STING on CRC cells are mediated by AMPK-mTOR pathway and STING regulates glucose uptake in CRC cells

This work, we conducted many bioinformatics analysis, including co-expression, GO, KEGG and protein-protein interaction analysis, to reveal the potential functions of STING in CRC. First, Using the STRING database, the PPI network containing 21 proteins was obtained (Fig. 3a). And, then we carried out GO/KEGG enrichment analysis on 21 genes (Fig. 3b, 3c). Our analysis shows that STING was involved in a series of biological processes of colorectal cancer. Meanwhile, the outcomes of cBioPortal indicated that STING is bound up with the mTOR in CRC (Fig. 3d). Combined with the above results, we put forward this hypothesis that STING may promote the progress of CRC through mTOR pathway. Transfection of HCT116 and SW480 cells with STING-siRNA inhibited STING expression (Fig. 3e). Meanwhile, AMPK, p-AMPK, mTOR, and p-mTOR expressions were detected (Fig. 3f). Western blot results showed that STING knockdown significantly inhibited AMPK-mTOR signaling, confirming the regulatory effect of STING. Collectively, this shows that STING promotes the occurrence and development of CRC cells by inhibiting AMPK
and activating the mTOR-related pathway. In the basis of our present experimental results, we preliminarily proved that STING may regulate the migration, proliferation, invasiveness and drug sensitivity of CRC cells by mediating the AMPK-mTOR pathway, which regulates a series of important functions, such as intracellular energy metabolism and biosynthesis. Firstly, AMPK and mTOR are intracellular energy receptors that balance the production, consumption, and synthesis of intracellular energy sources through negative feedback regulation to maintain homeostasis. Secondly, mTOR mediates some downstream signaling pathways and participates the metabolism of sugars, lipids, amino acids, and other substances. We questioned whether the increased glucose uptake observed in tumor cells is regulated by STING signaling. Compared with the control group, the glucose uptake of STING knockdown group was significantly lower (Fig. 3g). It suggests that STING is not only involved in regulating AMPK-mTOR pathway activity, but also regulates glucose uptake, which might further mediate CRC cell proliferation and metastasis.

**hsa-miR-193b-3p may be a key miRNA in ceRNA network**

Starting from the prediction results of TargetScan and ENCORI, we obtained 20 miRNAs (number of repetitions ≥ 7). Then, we used Incbase v.2 (PR score > 0.8) to predicted 105 lncRNAs which is related with these miRNAs. After that, we constructed a STING CeRNA network using Cytoscape (Fig. 4a, 4b). Through searching 20 miRNAs on ENCORI, results showed that in terms of differential expression and survival rate, only has-mir-193b-3p had obvious clinical significance (Fig. 4c, 4d).
STING affects the infiltration of immune cells in COAD and is a key factor in many tumors

The results of TIMER confirmed that in COAD, STING was bound up with B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and mDC cells infiltrating tumor tissues (Fig. 4e). Therefore, we speculate that there is a promising research direction, that is, STING affects the progression of colorectal cancer via regulating immune cell infiltration. Notably, Pan-cancer analysis showed that STING was potentially a common oncogene in many tumors (Fig. 4f-4h).

Discussion

Tumorigenesis of gastrointestinal malignancies is closely related to nonspecific inflammation resulting from the innate immune system aberrant activation [15]. Dysregulated innate immune responses mediated by STING predispose to colorectal carcinogenesis [16,17]. STING dysfunction may cause CRC and increase the susceptibility of melanoma cells to oncolytic viruses [16,18], indicating that STING could represent an adjunctive therapeutic target. Furthermore, human papillomavirus E7 and adenovirus E1A oncoproteins bind to STING and inhibit its function [19], suggesting that to some extent STING has a protective role against infections due to these oncogenic viruses. Moreover, STING expression is reduced in gastric cancer, and decreasing levels are related to the poor prognosis [20]. However, using TCGA database analysis, elevated expression of STING in CRC was confirmed. In an earlier study, STING served as a biomarker of overall survival after adjusting for
tumor stage and intratumoral CD8+ T-cell infiltration [6]. Furthermore, STING expression is upregulated in the consensus molecular subgroup-1 of CRC patients [5,21]. Therefore, we speculated that elevated STING expression in CRC tissues may portend a poor prognosis. To test this hypothesis, we evaluated STING expression levels in multiple patient-derived CRC tissue specimens and corresponding adjacent normal tissues, and found that elevated STING expression in cancer tissues. In addition, we associated STING expression levels with clinical indicators, and showed that high STING expression levels were related to advanced TNM stages of CRC.

Previous studies have demonstrated that STING-mediated immune pathways are involved in the tumorigenesis of many malignancies. Abnormal STING function significantly affects, cancer cell proliferation, metastasis, and antitumor immunity. Tumor drug sensitivity is enhanced by the recruitment and infiltration of immune effector cells stimulated by STING-mediated interferon (IFN) production triggered by increased cytoplasmic dsDNA induced by adjuvant chemotherapy [17,22-25]. However, the potential roles of STING-related pathways as therapeutic targets to inhibit tumorigenesis, enhance anti-tumor immunity, and reduce drug resistance in CRC deserve further investigation [26,27].

In the present study, we selected HCT116 and SW480 CRC cell lines with high STING expression levels and then downregulated STING expression via siRNA interference. We then used the constructed cell model to perform relevant cell function experiments. First, we found significantly decreased CRC cell proliferation after STING knockdown, which verified the hypothesis that elevated STING
expression promotes CRC cell proliferation. We also confirmed this result by using a clonogenic assay. In addition, the transwell assay indicated that STING knockdown reduced CRC cellular migration and invasiveness. Taken together, our results suggest that STING signaling significantly promotes CRC development.

STING signaling may also influence CRC drug resistance [21,28]. Our study suggests that STING knockdown improves the chemosensitivity of CRC cells to 5-FU. Notably, 5-FU activates the cGAS-STING pathway that produces type I IFN in CRC cells. Contrary to our results, Tian et al. reported that STING did not significantly change the lethal effect of 5-FU on colon cancer cells in vitro. The authors speculated that endogenous IFN expression by cancer cells was insufficient to achieve enhanced cytotoxicity in vitro [28].

STING-mediated signaling also inhibits tumor growth by activating the innate immune system, thereby upregulating downstream secretion of immune regulatory factors such as IFN and the consequent recruitment and infiltration of immune effector cells that include macrophages, dendritic cells, and CD8+ lymphocytes [17,18,21]. However, functional defects in STING-related pathways may inhibit IFN secretion in CRC cell lines [29-32]. However, recent study confirmed that CGAs acts on the prevention of colon cancer uniquely [33]. Our work revealed that the effect of STING on CRC cells may not be regulated by the classical innate immune pathway, suggesting that STING regulates the progression of CRC and affects drug sensitivity through IFN-independent signaling.

Our study demonstrated that STING overexpression is associated with markers of
advanced CRC. We constructed a STING knockdown CRC cell line and found that reduced STING expression was accompanied by decreased proliferation and migration and enhanced 5-FU sensitivity. However, STING-related signaling is often impaired in CRC. At the same time, other studies have disclosed that STING-mediated downstream antitumor immune responses activated by DNA damage enhance radiation- and chemotherapy-induced cytotoxicity [6,25,34]. In view of the above results, we propose that STING regulates the function of CRC cells through an IFN-independent pathway.

In this study, we first used GEO microarray data for GSEA to identify pathways that are significantly enriched after STING activation. Second, we focused on changes in mTOR-related pathway function. Combined with our previous findings [13,14,35], the present study demonstrates that STING activation upregulated the mTOR-related pathway. mTOR activation promotes intracellular metabolism, enhances protein synthesis, inhibits autophagy, and forms a negative feedback loop with AMPK [8]. Therefore, we suggest that STING regulates CRC cells through the AMPK-mTOR pathway. We found that AMPK function increased after STING downregulation, whereas mTOR function decreased, indicating that a series of high energy-consuming processes was inhibited in CRC cells [36]. At the same time, the effect of STING signal on glucose uptake was explored in CRC cells. The glucose uptake rate decreased with the down-regulation of STING, which confirmed that STING may regulate the energy metabolism. Thus, above results show that the regulation of STING is mediated by AMPK-mTOR pathway, which may change energy metabolism and affect proliferation,
migration and drug sensitivity.

We have to admit that there are some limitations in our current work. We only briefly describe the effect of mTOR pathway on cell energy homeostasis. In addition, glucose entry into cells is mediated by membrane transporters. The correlation between mTOR pathway and glucose uptake seems to be very small, but it can not be ignored that mTOR pathway is closely related to autophagy. As we all know, autophagy involves a variety of organelle membranes, and the renewal of various organelle membranes is inseparable from the cell membrane. The renewal of cell membrane must involve the dynamic changes of cell membrane proteins. More importantly, there is also an important link between autophagy and energy metabolism. There is no doubt that the most important mechanism of cell metabolism is glucose metabolism. Of course, the deepening of the mechanism is also the direction of our further attention and exploration in the future. More importantly, in vivo experiments and monitoring of important CRC-related indicators are warranted.

**Conclusion**

Our results reveal a new mechanism that links STING with AMPK-mTOR pathway in monitoring progression of colorectal cancer, and identifies therapeutic targets to improve clinical outcomes. It is worth noting that we reverse constructed STING CeRNA network in COAD and found a key miRNA, which can be used as the basis for in-depth research.
Acknowledgements

Huihui Yao, Suo Wang, Guoqiang Zhou and Diyuan Zhou contributed equally to this work. An earlier version of this manuscript has been uploaded to the website as a preprint (https://www.researchsquare.com/article/rs-1469741/v1) [37].

Authors’ contributions

SH, HY, and SW conceived and designed the study and drafted the manuscript. GZ, DZ, XZ, XS, GC, JC, GW, and LS collected, analyzed, and interpreted the experimental data. YY, WG and DW revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate
This study is approved by Biomedical Research Ethics Committee of the First Affiliated Hospital of Soochow University (Suzhou, China) (2021-NO:213) and in line with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients or their families.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References


Figure 1

(a) Relative expression of STING.
(b) Expression of TIM17 in CM49 based on sample type.
(c) Expression of TIM17 in CM49 based on total metastasis status.
(d) Expression of TIM17 in CM49 based on individual cancer stage.
(e) Tissue sections showing STING expression.
(f) Additional STING tissue images.
(g) Hematoxylin and eosin (H&E) staining.
(h) Western blot analysis of STING and Tubulin expression.
(i) Graph showing relative expression of STING and Tubulin.
(j) Comparison of STING/Tubulin expression in Cancer and Paracancer.
Figure 3
Figure 4

a) Venn diagram showing the overlap between TargetScan and ENCORI.
b) Network diagram representing gene interactions.
c) Box plot with comparison of two groups.
d) Survival curve with Kaplan-Meier estimator.
e) Heatmaps showing gene expression patterns.
f) Scatter plot with expression levels of TMEM173 across TCGA cancers.
Table 1: Association between STING and clinic-pathological factors in 32 patients with colorectal cancer.

<table>
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<th>Clinic parameters</th>
<th>Case No.</th>
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<th>P value</th>
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<tr>
<td></td>
<td></td>
<td>None or low</td>
<td>High</td>
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<tr>
<td>Total</td>
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<td>&lt;65</td>
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<td>≥65</td>
<td>18</td>
<td>8 (44.4%)</td>
<td>10 (55.6%)</td>
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<td>Gender</td>
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<tr>
<td>Male</td>
<td>19</td>
<td>7 (36.8%)</td>
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</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>4 (30.8%)</td>
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</tr>
<tr>
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<tr>
<td>Negative</td>
<td>21</td>
<td>9 (42.9%)</td>
<td>12 (57.1%)</td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>2 (18.2%)</td>
<td>9 (81.8%)</td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
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</tr>
<tr>
<td>I/II</td>
<td>21</td>
<td>8 (38.1%)</td>
<td>13 (61.9%)</td>
</tr>
<tr>
<td>III/IV</td>
<td>11</td>
<td>9 (81.8%)</td>
<td>2 (18.2%)</td>
</tr>
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* P < 0.05
Figure legends

**Figure. 1 STING expression in colorectal cancer (CRC) and adjacent tissues.**

(a, b) Analysis of the COAD GEO (GSE100179) and TCGA dataset, confirmed that the protein and mRNA of STING was increased significantly in CRC tissues. (c, d) The outcome of UALCAN confirmed that mRNA of STING was especially higher in patients with higher histological grade (poor differentiation) and more advanced clinical stage. (e) Hematoxylin-eosin staining of CRC tissue (200×, scale: 100 µm). (f, g) An immunohistochemical method was adopted to detect STING expression in 32 pairs of CRC and adjacent tissues (Fig. 1f scale: 100 µm, Fig. 1g scale: 50 µm). (h) IHC score of STING in 32 pairs of CRC and adjacent tissues: Paired samples t-test; IHC score analysis for tumor tissue with and without lymph node metastasis. (I, j) The expression level of STING in CRC and adjacent tissues was detected by Western blotting, and the gray value of cancer and adjacent tissues was analyzed quantitatively. \( P <0.05 \) was considered statistically significant. Note \( *P <0.05, \ **P <0.01, \ ***P <0.001. \)

**Figure. 2 STING promotes CRC tumor cell growth, migration, invasiveness, and drug sensitivity.**

(a) Detection of STING expression in CRC cell lines using western blotting; Quantitative analysis of STING expression in CRC cells. (b) Transfection efficiency of siRNA in CRC cells verified by fluorescent siRNA probe. (c) Verification of knockdown and overexpression efficiency using western blotting. (d, e) Twenty-four hours after transfection, numbers of migrating and invasive cells after treatment with NC-siRNA
NC and STING-siRNA = STING-KD were determined and recorded using transwell assays (three to five fields were taken for each group, and the results were analyzed quantitatively). (f) The CCK-8 assay was carried out to evaluate the proliferation ability of cells (NC-siRNA, and STING-siRNA) at 24, 48, and 72 h. (g) The absorbance of concentration in each group was measured after 24, 48, and 72 h using the CCK-8 assay for the CTL group, STING-siRNA group, and 5-fluorouracil-treated cells group, respectively, and was then analyzed and calculated (5-fluorouracil concentration: 2.5 µM). P <0.05 was considered statistically significant; * P <0.05.

Figure. 3 STING regulates biological characteristics through AMPK-mTOR pathway and glucose uptake in CRC cells.

(a-c) GSEA using gene sequencing data from the GEO datasets GSE129436 and 100179 (KEGG enrichment, tumor-associated pathways). (d) cBioPortal indicated that STING is closely related to the mTOR in CRC. (e) 48 hours after transfection of STING siRNA and the negative control siRNA, the expressions of p-mTOR, mTOR, p-AMPK, AMPK and STING were detected by Western blotting. (f) Quantitative analysis of the gray scale values of results from triplicates of Figure 3e. P <0.05 was considered statistically significant. Note * P <0.05, ** P <0.01, and *** P <0.001. (g) Glucose fluorescent probe to detect the glucose uptake of HCT116 and SW480 CRC cells. Average fluorescence intensity was quantified by randomly selecting 5 microscopic fields. (*, P<0.05)

Figure. 4 Bioinformatics predicted the relationship between STING and miRNA, and between STING and infiltrating immune cells.
(a, b) Using Cytoscape tool, we constructed the CeRNA network of STING. mRNA is represented by orange oval, miRNA is represented by green diamond, and IncRNA is represented by blue rectangle. (c, d) Based on ENCORI database, the expression of hsa-mir-193b-3p and the relationship between the expression of hsa-mir-149-3p and the overall survival rate were detected. (e) The correlation between STING expression and the infiltration of immune cells in COAD in TIMER database. (f-h) Pan cancer analysis shows that STING may be a common oncogene in a variety of cancers.