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Pathological expression of mitochondrial genome-derived circRNA SCAR/mc-COX2 and its ceRNA network in colorectal cancer: implications for clinical significance

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Abstract

Background Mitochondrial-encoded circular RNAs (mecciRNAs) are a newly discovered class of mitochondrial-encoded non-coding RNAs (mt-ncRNAs) that play important biological roles in the cell. This study aimed to examine the expression profile of SCAR/mc-COX2 (has_circ_0089762) in colorectal cancer (CRC) and its relationship with clinicopathological variables. Furthermore, to better understand SCAR/mc-COX2's functional role in CRC, we constructed a competing endogenous RNA (ceRNA) network.

Methods Quantitative real-time PCR (qRT-PCR) was employed to analyze the expression levels of SCAR/mc-COX2 in 40 pairs of CRC samples, consisting of 40 tumor samples and 40 adjacent non-tumoral samples from patients. The ceRNA regulatory network was constructed using online bioinformatics tools. Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) enrichment analysis were conducted using the Enrichr database.

Results The results demonstrated a significant decrease in SCAR/mc-COX2 expression in tumor tissues compared to adjacent non-tumoral tissues (p-value<0.05). In another finding, a significant relationship was observed between pathological T staging and the expression status of SCAR/mc-COX2 (p-value=0.02). Additionally, the Receiver Operating Characteristic (ROC) curve analysis revealed that SCAR/mc-COX2 had an area under the curve (AUC) of 0.77, with 80% sensitivity and 75% specificity. Finally, a ceRNA regulatory network including SCAR/mc-COX2, 5 miRNA, and 9 mRNAs was found.

Conclusion These findings suggest that SCAR/mc-COX2 may act as a tumor suppressor in CRC, and its dysregulation could play a crucial role in the pathophysiology of this cancer. The significant association with pathological T staging and its robust diagnostic performance (AUC = 0.77, sensitivity = 80%, specificity = 75%) highlight its potential

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as a novel biomarker for CRC detection and prognosis. Further functional studies are required to elucidate its precise role in CRC tumorigenesis and clinical applicability.

Keywords SCAR/mc-COX2, MecciRNA, Colorectal cancer, CeRNA Network, Metabolic Reprogramming

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide [1]. In 2022, there were approximately 1.9 million new cases of CRC and 903,859 cancer-related deaths, accounting for roughly 9.6% of all cancer cases and 9.3% of cancer-related fatalities [1]. This cancer occurs in two forms: rare hereditary form and common sporadic type. Sporadic colorectal cancer (CRC) has a complex etiology, involving various genetic and environmental factors that promote tumorigenesis in colonic tissue cells by causing pathological molecular alterations in the cellular genome and transcript [2–4]. The revelation of the formation and development of these molecular changes not only helps to track the complicated CRC carcinogenic processes accurately, but also leads to the identification of more efficient diagnostic, prognostic, and therapeutic targets [5].

Aberrant genetic alterations drive CRC tumor-related phenotypes by affecting diverse cell signaling pathways, thereby inducing abnormal cellular events that contribute to the development of the tumor microenvironment (TME) [6]. TME supports tumor characteristics such as proliferation, invasion, progression, metastasis, and escape from apoptosis by providing settings for the execution of demands and programs of CRC tumor cells [7]. One of the outstanding hallmarks of TME is the mitochondrial dysfunction for reprogramming of tumor metabolism called the Warburg effect, in which tumor cells limit the production of energy from various metabolic mediators by the Krebs cycle and OXPHOS (oxidative phosphorylation) while inducing the production of energy from glucose by the glycolysis pathway [8]. This altered mitochondrial metabolism allows the tumor cells to enter the resulting glycolytic metabolites into the biosynthetic pathways that create macromolecules and organelles necessary for the production of new cells [9]. Furthermore, it facilitates the generation of reactive oxygen species (ROS), which act as a mutation-inducing tool as well as stimulators of oncogenic pathways and inhibitors of tumor suppressive pathways [10]. Moreover, altered metabolic signal messages in CRC cells are significantly influenced by the fusion-fission dynamics of mitochondria, enhancing their metabolic reprogramming, leading to substantial metastasis and chemoresistance [11].

Various research findings have revealed that in addition to the direct involvement of pathologic changes in nuclear genome DNA and its transcriptome in promoting metabolic reprogramming [12], deviations of mitochondrial DNA (mtDNA) and mitochondrial transcriptome have a key role in this tumor-related metabolic hallmark [13, 14]. Likewise, mitochondrial dysfunction and mtDNA abnormalities, including point mutations, deletions, and copy number changes, significantly contribute to the development and clinicopathology of CRC [15, 16]. It was previously thought that the mitochondrial transcriptome was limited to only 22 tRNAs, 2 rRNAs, and 13 proteins, but recent research has shown that mtDNA expresses various groups of mitochondrial-encoded noncoding RNAs (mt-ncRNAs) that control important functions of the mitochondria and even the nucleus [17–19].

Recently reported classes of mt-ncRNAs are mitochondria-encoded circular RNAs (mecciRNAs), whose biological roles are mainly considered unknown [20, 21]. CircRNAs are a recently discovered class of ncRNAs produced by back-splicing with a closed circular structure [22]. CircRNAs have significant effects on the regulation of gene expression by binding to RNA binding proteins (RBPs) and functioning as competing endogenous RNAs (ceRNAs) via the circRNA-microRNAs (miRNA)-mRNA axis [5, 23]. Also, a robust body of evidence demonstrates a strong link between the dysregulation of circRNAs and the formation and development of CRC [5, 24–27]. Recent research has explored the role of mecciRNAs in the development and progression of cancer, shedding light on their biological functions within this context. For example, studies have demonstrated that the expression levels of mecciND1 and mecciND5, two mecciRNAs originating from the *ND1* and *ND5* genes, are significantly elevated in hepatocellular carcinoma tissues [20]. These mecciRNAs are known to play crucial roles in both normal physiological processes and pathological conditions. Unlike many nuclear-encoded circRNAs that have been extensively studied in CRC, the biological significance of mecciRNAs remains largely unexplored.

Steatohepatitis-associated circRNA ATP5B regulator (SCAR), also referred to as mc-COX2 (hsa-circ-0089762), is a mecciRNA encoded by the *COX2* gene on the mitochondrial light strand [28]. Interestingly, new evidence is mounting to suggest that SCAR/mc-COX2 plays crucial cellular roles in different tissues, and its aberrations are linked to various diseases, including chronic lymphocytic

leukemia (CLL), nonalcoholic steatohepatitis (NASH), dilated cardiomyopathy, and diabetic retinopathy [29–32]. For example, upregulation of SCAR/mc-COX2 has been observed in the plasma and exosomes of CLL patients, where it regulates mitochondrial function, cell proliferation, and cell death [29]. Additionally, researchers discovered that this mecciRNA has lower expression levels in the fibroblasts of individuals with NASH. They found that SCAR/mc-COX2 inhibits the release of mitochondrial ROS (mROS) and fibroblast activation by binding to ATP5B and blocking the mitochondrial permeability transition pore (mPTP) [30]. However, the role of SCAR/mc-COX2 in CRC remains completely uncharacterized. Given the crucial involvement of mitochondrial dysfunction in CRC progression, investigating the pathological expression and potential regulatory role of SCAR/mc-COX2 may provide novel insights into CRC tumorigenesis and open new avenues for biomarker discovery and therapeutic targeting. Therefore, in this study, we comparatively examined the clinical significance of SCAR/mc-COX2 expression levels in tumor tissue samples and adjacent non-tumoral tissue, as well as its relationship with clinicopathological variables. Additionally, we constructed the SCAR/mc-COX2-related ceRNA network using bioinformatics approaches.

Materials and methods

Patient samples

For this case-control study, 40 paired samples, including CRC tissue samples and adjacent non-tumoral tissues, were obtained from Iran National Tumor Bank (Imam Khomeini Hospital, Tehran, Iran), which collected informed consent from all patients before participating. The experimental procedures were approved by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.MED.REC.1402.018). None of the patients had undergone any preoperative treatments, such as radiation or chemotherapy, before surgery.

Gene expression analyses

According to the manufacturer's instructions, total RNA was isolated from tumor and adjacent non-tumor tissue samples using RNSol H reagent, (ROJE Technologies, Tehran, Iran). Gel electrophoresis and the absorption ratio at 260–280 nm (A260/280) with a Nanodrop were used to measure the quality and quantity of the extracted RNAs, respectively. The Easy cDNA Synthesis Kit (Parstous, Mashhad, Iran) was used to create cDNA, following the manufacturer's instructions. Quantitative Real-time PCR was conducted using specific primers [29, 33] for SCAR/mc-COX2 and *ACTB* (as an internal control) with ExcelTaq 2X Q-PCR Master Mix (SMOBIO), and run on the Rotor-Gene 6000 (Qiagen, Hilden, Germany). Additionally, the calibrator sample was used as an external control and *ACTB* as an internal control to normalize the expression levels of SCAR/mc-COX2. The calibrator served as a reference sample to lower variability between runs. Every qRT-PCR reaction was carried out in duplicate to reduce intra-run variability. Note that samples with a Ct difference greater than 0.5 were repeated. The PCR cycling conditions for the genes included an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 59°C and 56°C for *ACTB* and SCAR/mc-COX2, respectively, for 30s, and extension at 72°C for 30s. The identity of PCR products was further verified by gel electrophoresis on a 2% agarose gel. The fold change of SCAR/mc-COX2 was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics 27, and graphs were created using Excel software. A p-value of <0.05 was considered significant. Since the Kolmogorov-Smirnov test rejected the normality of data distribution, the Wilcoxon rank test was utilized to determine the difference in relative expression of SCAR/mc-COX2 between tumors and adjacent non-tumoral tissues. Mann-Whitney and Kruskal-Wallis tests examined the association between the SCAR/mc-COX2 fold change and clinicopathological data. Subsequently, the SCAR/mc-COX2 fold change was separated into two groups, low expression, and high expression, based on the median. The chi-square test was used to analyze the difference between these groups. The diagnostic values were estimated using the Receiver Operating Characteristic (ROC) curve and the area under the ROC curve (AUC).

Bioinformatic analysis

Construction of the SCAR/mc-COX2-related ceRNA network

To construct the ceRNA network mediated by SCAR/mc-COX2, we identified SCAR/mc-COX2-miRNA and SCAR/mc-COX2- RBPs interactions using the CircInteractome online database (https://circinteractome.nia.nih.gov/) [34]. In the next step, we employed TargetScan (https://www.targetscan.org/vert_80/) and miRTarBase (https://mirtarbase.cuhk.edu.cn/) to identify the target mRNAs of these miRNAs and then found the common mRNAs from these two databases [35, 36]. It is important to note that TargetScan forecasts the biological targets of miRNAs, while miRTarBase provides data on these interactions that have been confirmed experimentally. We downloaded the series matrix files for two datasets from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to strengthen the study and

refine the mRNA and miRNA data obtained in the previous step. These datasets include the expression profiles of miRNA (GSE126093) and mRNA (GSE126092) in CRC tissues and their corresponding normal tissues from the same samples via a SuperSeries (GSE126095). Notably, the GSE126093 and GSE126092 datasets each contain 10 CRC tissue samples and 10 adjacent normal tissue samples. Importantly, both datasets were derived from the same cohort of subjects participating in the same trial, ensuring consistency across analyses. Their selection was primarily based on the inclusion of CRC and adjacent normal tissue samples, which closely align with the conditions of our study. We then identified common mRNAs and miRNAs and calculated the correlation and *p*-values for the expression between miRNAs and their target mRNAs in the GSE126093 and GSE126092 datasets. Finally, we considered miRNA-mRNA interaction with two criteria: significant (FDR adj-*p*-value <0.05) and negative ($-1 < r < 0$) correlation between miRNA and mRNA expression, and predicted in the above target prediction tools. Protein-protein interaction (PPI) predictions were made using the STRING database (https://string-db.org/) with high confidence (more than 0.7) without considering the neighbors between proteins. The network was visualized using Cytoscape software (version 3.10.2) [37]. Figure 1 displays an overview of the bioinformatic analysis performed in the present study.

SCAR/mc-COX2-ceRNA mediated signaling pathway prediction

We used the Enrichr web-based tool (https://maayanlab.cloud/Enrichr/) to perform enrichment analysis to determine mRNAs' involvement in the ceRNA network about Gene Ontology (GO) in biological processes (BP), molecular function (MF), and signaling pathways (Kyoto Encyclopedia of Genes and Genomes -KEGG) [38]. We presented significant GOs and pathways with adj-*p*-value < 0.05.

Expression validation of mRNA involving in the predicted ceRNA networks

Gene expression profiling interactive analysis (GEPIA; http://gepia.cancer-pku.cn/) is an online database that was developed utilizing data from The Cancer Genome Atlas (TCGA) program and the Genotype-Tissue Expression (GTEx) project [39]. A differential expression boxplot in GEPIA was used to compare the expression of predicted mRNAs in the SCAR/mc-COX2 ceRNA network between tumor samples of colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ), as well as their corresponding normal samples. The adj-*p*-value threshold was 0.01 and the log2FC cutoff was 1.

Results

Clinicopathological characteristics of CRC patients

The study population consisted of 40 CRC patients who underwent surgical resection. The clinicopathological characteristics of these patients are shown in Table 1. This study included 19 patients (47.5%) over and 21 patients (52.5%) under the age of 60. The average age of the 12 females (30%) and 28 males (70%) in the investigated population was 59.33 years (range: 26–93 years) at the time of diagnosis. The primary tumor was found in the colon in 65% of patients and the rectum in the remaining 35%. Of the patients, 32 (80%) were in stages III–IV, while 8 (20%) were in stages I–II. Furthermore, lymphatic invasion was observed in 26 (65%) patients, whereas 14 (35%) exhibited no lymphatic invasion.

The expression levels of SCAR/mc-COX2 in tumors and non-tumoral adjacent tissues

The statistical analysis of the qRT-PCR data demonstrated a significant downregulation of SCAR/mc-COX2 in tumor tissues (median= 0.53) compared to the adjacent non-tumoral tissues (median= 0.98; *p*-value= 0.002; Fig. 2).

Clinical significance and diagnostic value of SCAR/mc-COX2

The clinical and diagnostic value of SCAR/mc-COX2 as a potential biomarker was evaluated using ROC curve analysis. The results indicated that SCAR/mc-COX2 could serve as a biomarker for CRC diagnosis and prognosis (AUC= 0.77; *p*-value< 0.0001; Fig. 3), with 80% sensitivity and 75% specificity at a cutoff value of 0.78. The correlation between clinicopathological characteristics and SCAR/mc-COX2 expression was analyzed, revealing a significant association between the pathological T staging and SCAR/mc-COX2 down-regulation. Specifically, the T3-T4 group exhibited lower SCAR/mc-COX2 expression than the T1-T2 group (median 0.49 vs. 1.58; *p*-value= 0.02; Table 2). Furthermore, we classified CRC patients into low and high groups according to the median SCAR/mc-COX2 fold change. The comparison of clinicopathological variables between these two groups approved a significant relationship with the pathological T staging (*p*-value= 0.01; Table 3).

Data from bioinformatic analysis

Construction of the SCAR/mc-COX2-mediated ceRNA regulatory network

The hsa-circ-0089762-miRNA-mRNA network was constructed using 1 mecciRNA, 5 nuclear miRNAs (hsa-miR-1249, hsa-miR-433, hsa-miR-487a, hsa-miR-554 and hsa-miR-938), and 9 nuclear mRNAs

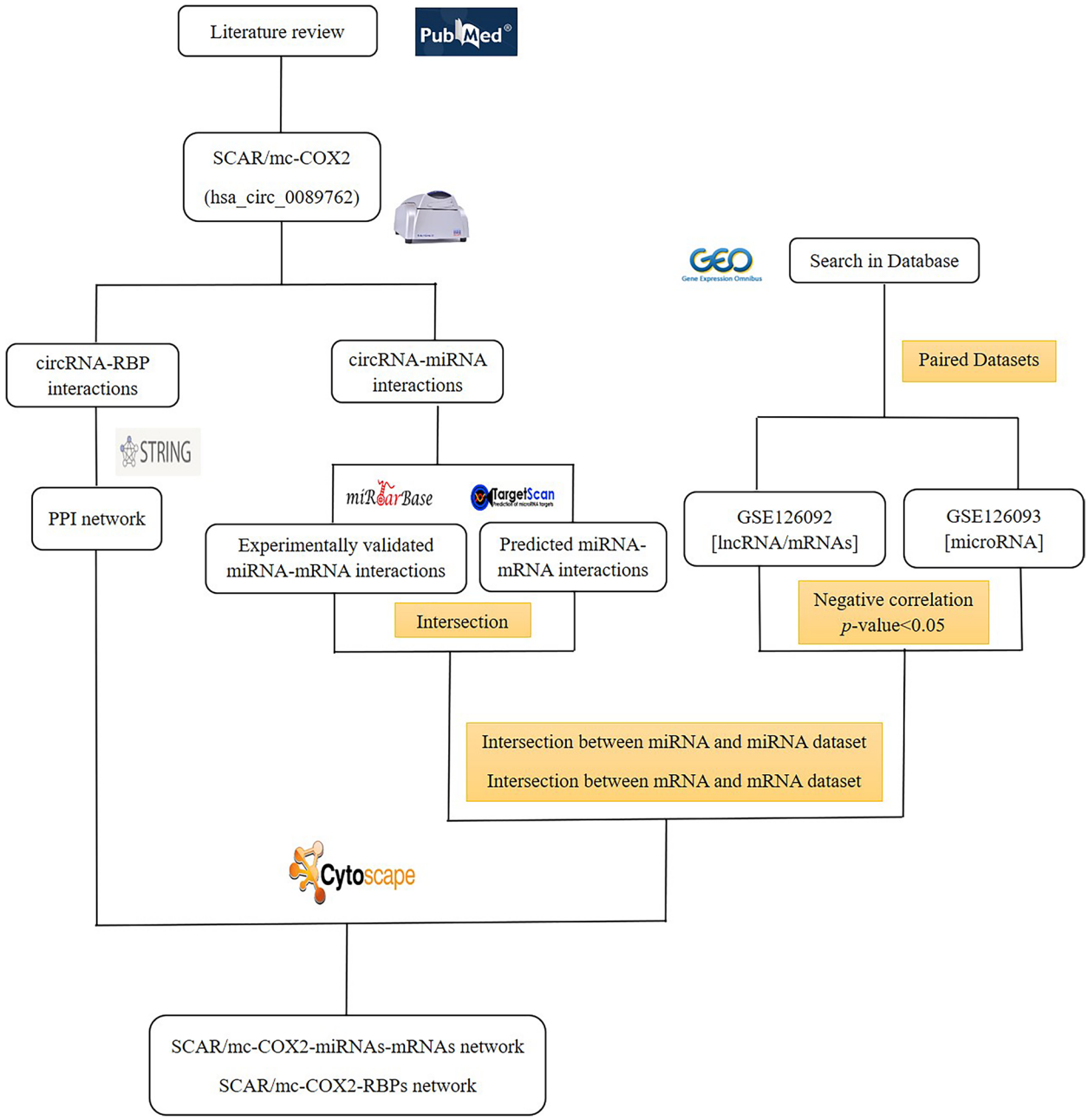


Fig. 1 Workflow of the study

(PLCG2, SOS1, DTWD2, CPEB4, BTRC, FAM107A, MGLL, FOXJ3 and FAM120AOS). Additionally, the interaction between SCAR/mc-COX2 (hsa-circ-0089762) and RBPs (AGO1, AGO2, DGCR8, EIF4A3, FMR1, IGF2BP1, IGF2BP2, IGF2BP3, LIN28A, LIN28B, METTL3, and TNRC6A), as well as their PPI network obtained from STRING, which is illustrated using Cytoscape software (version 3.10.2) (Fig. 4). As shown in Fig. 4, it was predicted that SCAR/mc-COX2 regulates these nuclear mRNAs through its ceRNA activity, and the relevant axes are as SCAR/mc-COX2/hsa-miR-938/FOXJ3, MGLL, FAM107A, FAM120AOS, SCAR/mc-COX2/hsa-miR-554/BTRC, SCAR/mc-COX2/hsa-miR-487a/CPEB4, SCAR/mc-COX2/hsa-miR-433/SOS1, DTWD2, and SCAR/mc-COX2/hsa-miR-1249/PLCG2.

Table 1 Demographic and clinicopathological parameters of CRC participants

Characteristics		Count	Column N %
Age	<60	21	52.5%
	>60	19	47.5%
Sex	F	12	30.0%
	M	28	70.0%
Site of primary tumor	Colon	26	65.0%
	Rectum	14	35.0%
Grade	I	10	25.0%
	II	20	50.0%
	III	10	25.0%
Lymphatic invasion	Yes	26	65.0%
	No	14	35.0%
Vascular invasion	Yes	28	70.0%
	No	12	30.0%
Pathological T staging	T1-T2	5	12.5%
	T3-T4	35	87.5%
Pathological N staging	N0	8	20.0%
	N1-N2	32	80.0%
Clinical metastasis	M0	38	95.0%
	M1	2	5.0%
Stage	I-II	8	20.0%
	III-IV	32	80.0%
Smoking	Non-smoker	32	80.0%
	Smoker	8	20.0%

KEGG pathway enrichment analysis and GO functional annotation nuclear mRNA members of SCAR/mc-COX2-mediated ceRNA network

We used the Enrichr web-based tool to perform GO and KEGG enrichment analysis for 9 mRNAs in the ceRNA network. GO analysis showed that these mRNAs are significantly involved in biological processes such as the Fc-epsilon receptor signaling pathway (GO:0038095; p-value= 0.00004), positive regulation of I-kappaB phosphorylation (GO:1903721; p-value= 0.002), and positive regulation of interleukin-23 production (GO:0032747; p-value= 0.00) (Fig. 5a). The most significant terms for MF were phospholipase activity (GO:0004620; p-value= 0.0005), acylglycerol lipase activity (GO:0047372; p-value= 0.006), and phosphatidylinositol phospholipase C activity (GO:0004435; p-value= 0.008) (Fig. 5b). Furthermore, the results of the KEGG pathway analysis suggested that these mRNAs contribute to the pathophysiology of glioma (p-value= 0.0004) and non-small cell lung cancer (p-value= 0.0004), as well as other pathways, including the ErbB signaling pathway (p-value= 0.0006) and B cell receptor signaling pathways (p-value= 0.0005) (Fig. 5c).

GEPIA-based expression validation of nuclear mRNAs involved in SCAR/mc-COX2-mediated ceRNA network

Box plots based on the TCGA dataset were retrieved from the GEPIA online server to verify the differential

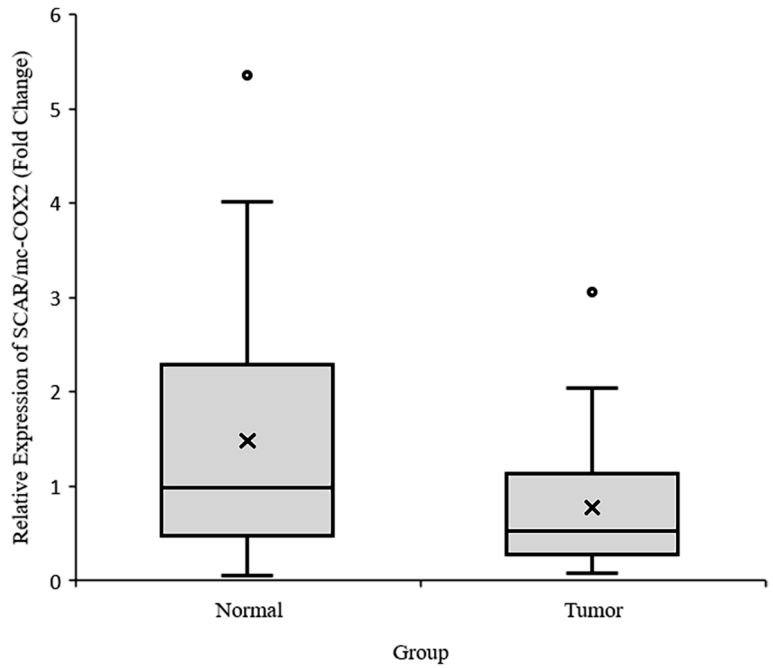


Fig. 2 Relative expression level of SCAR/mc-COX2 in CRC samples (n=40) compared to adjacent normal samples (n=40). The paired sample t test was used to compare the level of SCAR/mc-COX2 expression. SCAR/mc-COX2 expression was significantly decreased (p-value= 0.002) in tumor tissues compared to normal tissues

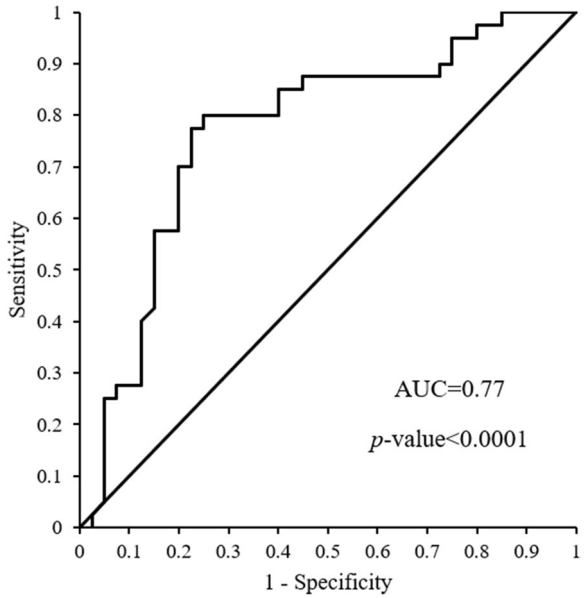


Fig. 3 ROC curve analysis on the relative expression level of SCAR/mc-COX2 to ascertain its biomarker value to distinguish tumors from normal samples

expression of 9 nuclear mRNAs in the ceRNA network. The results indicated that *FAM107A*, *MGLL*, and *PLCG2* genes exhibited significant downregulation in COAD and READ samples compared to normal samples (|Log2FC|>1; *p*-value<0.01) (Fig. 6 a, b, c, respectively) in line with the expression data of SCAR/mc-COX2 in CRC found by the present study.

Discussion

The normal metabolic function of mitochondria plays key roles in the control of apoptosis, cellular metabolism, metabolite synthesis, energy generation, and cellular homeostasis [40]. Extensive evidence obtained in recent years has revealed that mitochondrial defects and their resulting altered metabolism significantly affect various hallmarks of tumorigenesis development, including initiation, progression, drug resistance, angiogenesis, and metastasis in various cancers, especially in CRC cancer [11, 41]. Therefore, targeting this organelle has been proposed as a special therapeutic target in cancer therapy [41–43]. Apart from the important mutational defect of the mitochondrial genome in cancer, recent research has revealed that the mitochondrial transcriptome, especially its non-coding part including

Table 2 Association between the clinicopathological and demographic features of CRC patients and SCAR/mc-COX2 expression

Characteristics		SCAR/mc-COX2				*p*-value
		Number	Mean	SD	Median	
Age	<60	21	.63	.52	.52	0.35
	>60	19	.93	.80	.76	
Sex	F	12	.78	.55	.69	0.72
	M	28	.77	.73	.51	
Site of primary tumor	Colon	26	.65	.55	.46	0.1
	Rectum	14	1.01	.83	.69	
Grade	I	10	.67	.46	.56	0.94
	II	20	.78	.74	.49	
	III	10	.87	.77	.50	
Lymphatic invasion	Yes	26	.73	.72	.47	0.29
	No	14	.86	.60	.70	
Vascular invasion	Yes	28	.73	.70	.47	0.3
	No	12	.87	.62	.70	
Pathological T staging	T1-T2	5	1.34	.50	1.58	**0.02***
	T3-T4	35	.69	.66	.49	
Pathological N staging	N0	8	.85	.59	.76	0.36
	N1-N2	32	.76	.70	.49	
Clinical metastasis	M0	38	.74	.66	.51	0.1
	M1	2	1.50	.69	1.50	
Stage	I-II	8	.85	.59	.76	0.36
	III-IV	32	.76	.70	.49	
Smoking	Non-smoker	32	.72	.57	.52	0.56
	Smoker	8	1.00	1.02	.53	

Table 3 Association between the clinicopathological and demographic features of CRC patients and SCAR/mc-COX2 expression, according to dividing fold changes into two groups of high and low expressions

Characteristics		SCAR/mc-COX2				*p*-value
		Low		High		
		Count	Column N %	Count	Column N %	
Age	<60	11	55.0%	10	50.0%	0.75
	>60	9	45.0%	10	50.0%	
Sex	F	5	25.0%	7	35.0%	0.49
	M	15	75.0%	13	65.0%	
Site of primary tumor	Colon	14	70.0%	12	60.0%	0.5
	Rectum	6	30.0%	8	40.0%	
Grade	I	3	15.0%	7	35.0%	0.3
	II	12	60.0%	8	40.0%	
	III	5	25.0%	5	25.0%	
Lymphatic invasion	Yes	15	75.0%	11	55.0%	0.18
	No	5	25.0%	9	45.0%	
Vascular invasion	Yes	16	80.0%	12	60.0%	0.16
	No	4	20.0%	8	40.0%	
Pathological T staging	T1-T2	0	0.0%	5	25.0%	**0.01***
	T3-T4	20	100.0%	15	75.0%	
Pathological N staging	N0	2	10.0%	6	30.0%	0.11
	N1-N2	18	90.0%	14	70.0%	
Clinical metastasis	M0	20	100.0%	18	90.0%	0.14
	M1	0	0.0%	2	10.0%	
Stage	I-II	2	10.0%	6	30.0%	0.11
	III-IV	18	90.0%	14	70.0%	
Smoking	Non-smoker	16	80.0%	16	80.0%	1
	Smoker	4	20.0%	4	20.0%	

the circular mt-ncRNAs, is also disturbed in cancer in parallel with the nuclear transcriptome abnormality and dictates various abnormal processes in tumor cells [20, 28, 29, 44].

In this study, we investigated the expression level of a key mecciRNA, SCAR/mc-COX2 (hsa-circ-0089762), in tumor tissues of CRC patients. In addition, we clinically evaluated the relationship of its altered expression with the clinicopathological characteristics of the patients. Finally, to understand the possible function of this mitochondrial circRNA in CRC cancer, we also analyzed its regulatory role in the form of ceRNA regulatory networks using databases and bioinformatic tools. Our results demonstrated that SCAR/mc-COX2 was significantly downregulated in tumor tissues compared to non-tumoral samples. Moreover, there was a significant correlation between its reduced expression and higher pathological T stages in CRC patients. Remarkably, our study suggests that SCAR/mc-COX2 with an AUC of 0.77 has good biomarker potential in CRC based on the findings of the ROC curve analysis.

Therefore, this aberrant expression can be a clue about the role of SCAR/mc-COX2, especially acting as a tumor suppressor, in the development of CRC as well as its physiopathology. Interestingly, our bioinformatic evaluations using the construction of a ceRNA network to identify potential functions of this mecciRNA revealed potential hsa-circ-0089762-mediated ceRNA axes including hsa-circ-0089762/hsa-miR-938/FOXJ3, MGLL, FAM107A, FAM120AOS, hsa-circ-0089762/hsa-miR-554/BTRC, hsa-circ-0089762/hsa-miR-487a/CPEB4, hsa-circ-0089762/hsa-miR-433/SOS1, DTWD2, and hsa-circ-0089762/hsa-miR-1249/PLCG2. However, the verified data from the GEPIA online server found only a significant decreasing pattern for *FAM107A*, *MGLL*, and *PLCG2* expression in COAD and READ samples compared to normal samples. Of these, it seems that SCAR/mc-COX2-mediated ceRNA activity is more likely to participate in the pathogenesis of CRC via involving these three nuclear mRNAs and their relevant miRNAs, including hsa-miR-938 and hsa-miR-1249. In addition, the resulting data from KEGG pathway analysis revealed

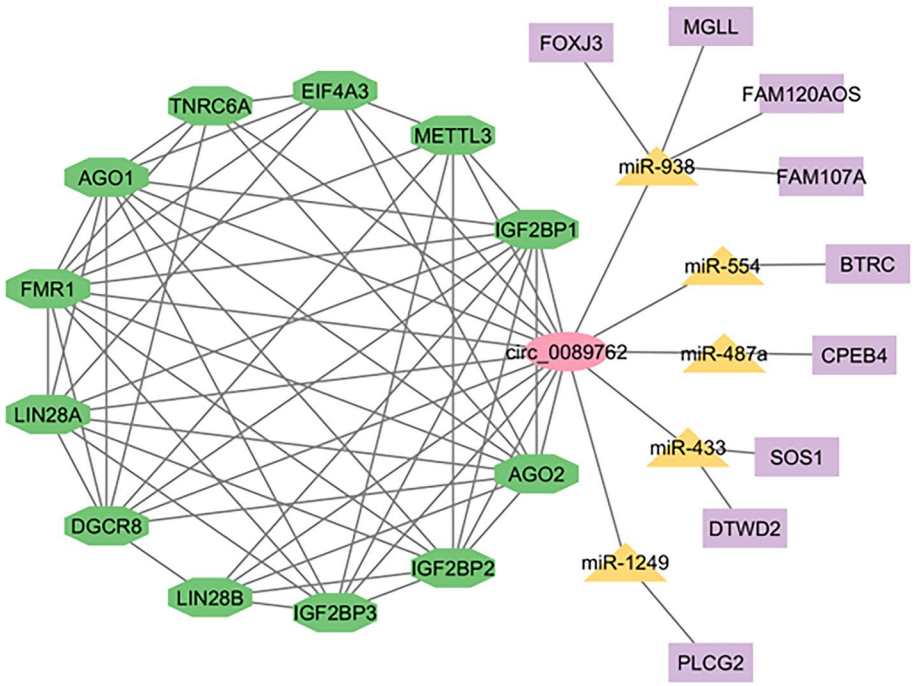


Fig. 4 The SCAR/mc-COX2-mediated ceRNA regulatory network as well as the association of SCAR/mc-COX2 with RBPs. Pink oval indicates SCAR/mc-COX2 mecciRNA, yellow triangle indicates miRNA, purple rectangle indicates mRNA, and green octagon indicates RPB

that these mRNA genes execute their functions through the regulation of some signaling pathways. How and by what mechanisms SCAR/mc-COX2 interacts with nuclear mRNAs and miRNAs remains unknown, however, evidence shows that mitochondrial non-coding RNAs, including mecciRNAs like SCAR/mc-COX2, can transport to the cytoplasm and nucleus to have extra-mitochondrial activity, including miRNA sponge function [31, 45].

As found in our results, miR-1249 targets PLCG2, while miR-938 targets both MGLL and FAM107A. It has been previously found that hsa-miR-938 is overexpressed in CRC tumor cells and tissue and functionally induces cell proliferation in this cancer by suppressing PHLPP2 [46]. Therefore, relying on this result and our bioinformatic data, it can be assumed that the interplay between SCAR/mc-COX2 and miR-938 may play a critical role in developing CRC through special molecular pathways. Our bioinformatic analysis also identified miR-1249 as an oncomiR target of SCAR/mc-COX2. Although this finding is contrary to the data reported in CRC, which indicates its tumor suppressor activity [47], this issue may be due to different molecular interactions resulting from the interaction of mitochondrial-specific transcriptome with nuclear transcriptome which can form completely different abnormal gene expression regulatory networks during CRC tumorigenesis. However, evidence is also available on its overexpression during tumorigenesis of other cancers [48, 49]. For example, Zhang et al. demonstrated that miR-1249 expression is significantly increased in gastric cancer cell lines and it suppresses apoptosis while promoting cell proliferation. It was also indicated that miR-1249 affects gastric cancer progression by sponge and regulation of *GNA11* expression [49]. Shu et al revealed that miR-1249 expression level increases in hepatocellular carcinoma and its knockdown inhibits cell proliferation, colony formation, and cell invasion [48]. Additionally, the significant decrease in mRNA expression of *FAM107A*, *MGLL*, and *PLCG2* observed in COAD and READ samples, compared to normal samples, not only confirms the importance of these protein-encoding genes in CRC pathogenesis but also emphasizes the value of the SCAR/mc-COX2 ceRNA network. In literature, miR-146b-3p can function as an oncogene by directly targeting the *FAM107A* gene, which promotes the proliferation, migration, and invasion of CRC cells while inhibiting their apoptosis [50]. The clinical significance of miR-938 and miR-1249 in cancer pathogenesis, coupled with the observed decrease in *FAM107A*, *MGLL*, and *PLCG2* expression in CRC, suggests that future research should focus on investigating the miR-938-mRNA (FAM107A or MGLL) and miR-1249-PLCG2 axes. This could provide a better understanding of the role of the SCAR/mc-COX2 pathway in CRC.

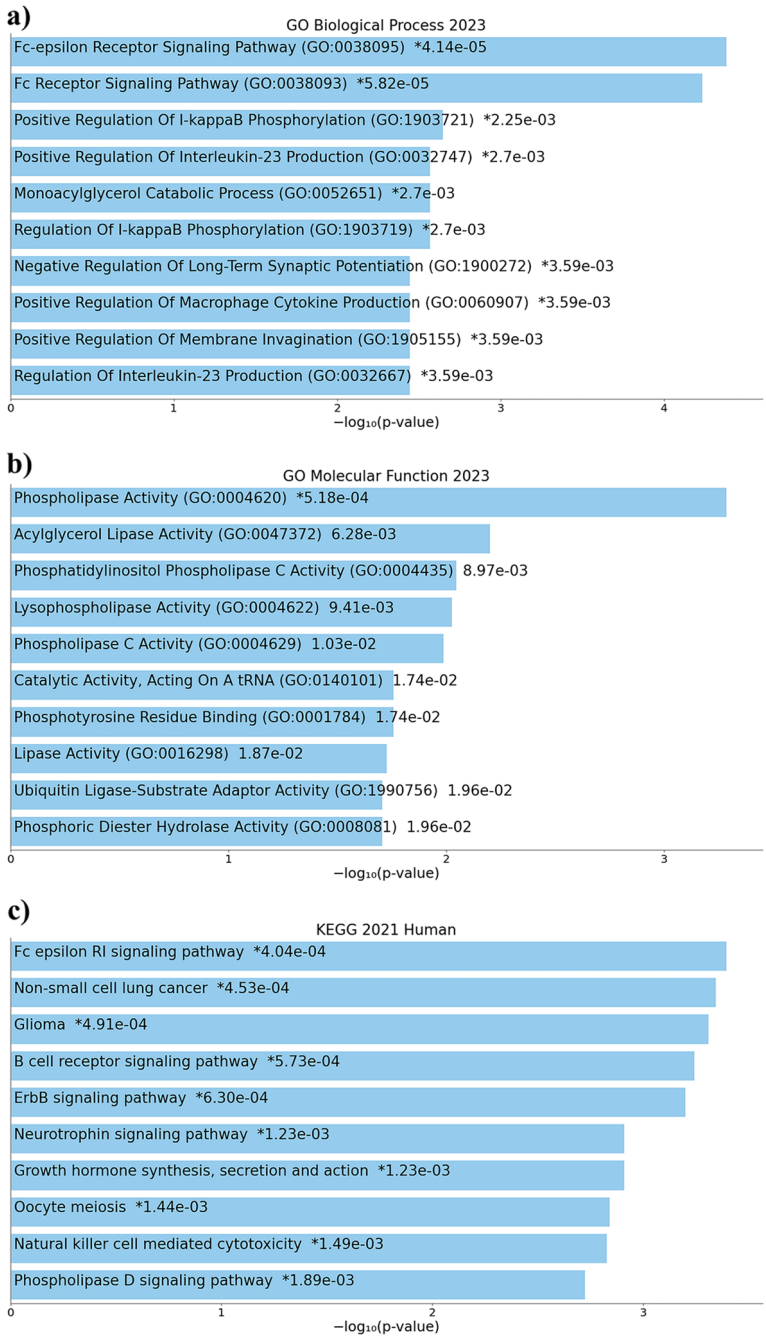
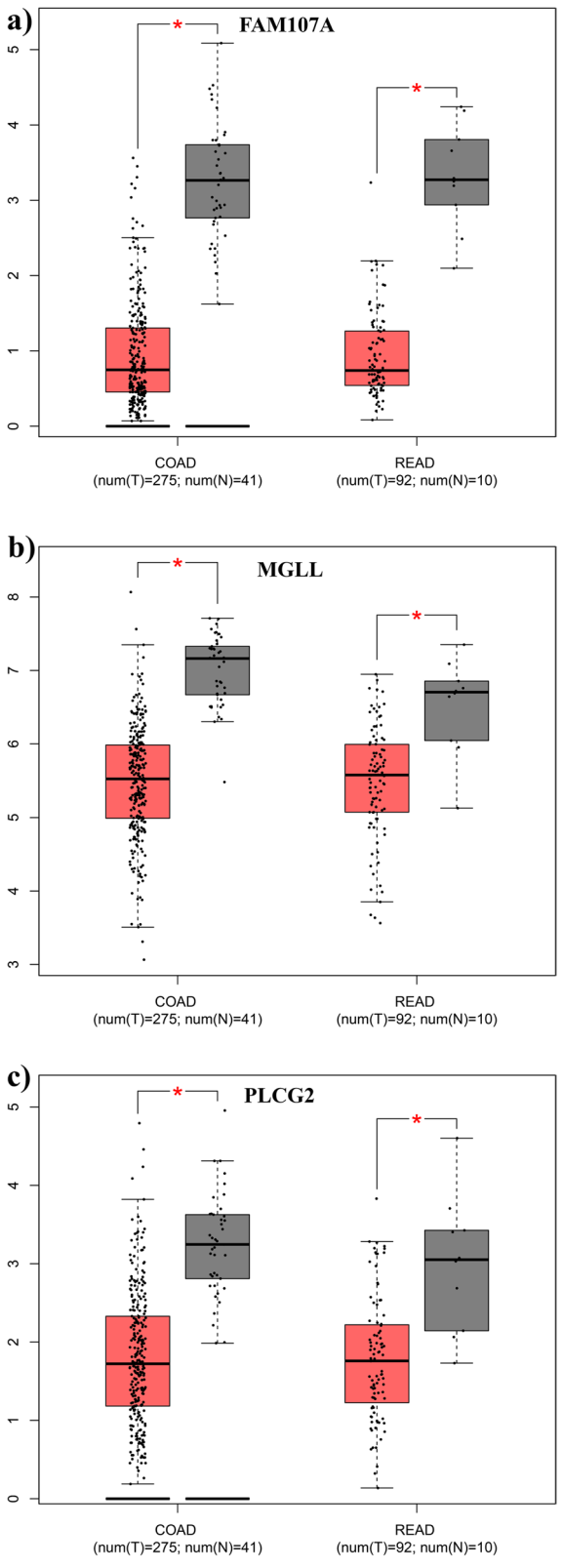


Fig. 5 GO and KEGG pathway enrichment analysis. Data obtained from Enrichr online database for (**a**) Biological Processes (BP), (**b**) Molecular Function (MF) and (**c**) KEGG pathway

In addition, we bioinformatically showed that SCAR/mc-COX2 could be involved in the pathogenesis of CRC through its protein sponge activity by interacting with some RBPs such as AGO1, AGO2, DGCR8, EIF4A3, FMR1, IGF2BP1, IGF2BP2, IGF2BP3, LIN28A, LIN28B, METTL3, and TNRC6A. Notably, several of these RBPs, such as IGF2BP1, METTL3, FMR1, and Lin28A, were shown to have significantly higher expression in CRC samples compared to normal samples, according to multiple research studies [51–54]. This observation may also be the clue that the protein interaction activity can be one of the biological roles of SCAR/mc-COX2, whose defect can be linked to CRC tumorigenesis through casing a deviation in numerous molecular regulating pathways.



◀ **Fig. 6** Validation of the differential expression of mRNAs in COAD and READ samples compared to normal TCGA dataset samples using the GEPIA online server. **a** FAM107A (**b**) MGLL (**c**) PLCG2. Red boxes represent tumor samples and gray boxes represent normal samples; * indicates *p*-value<0.01

It should be noted that previous studies in recent years have explored the role of SCAR/mc-COX2 in the pathogenesis of various human diseases, including cancer [29–32]. For example, in line with our findings, the expression profiling of circRNAs in the plasma of patients with esophageal squamous cell carcinoma (ESCC) showed that the plasma expression level of hsa-circ-0089762 is significantly reduced compared to the plasma of healthy controls [55]. While the study conducted by Wu et al. demonstrated that SCAR/mc-COX2 is significantly overexpressed in plasma samples and exosomes of CLL patients compared to normal samples. They also observed that the elevated expression of this mecciRNA was positively associated with CLL progression, increased leukemogenesis, and lower patient survival. Considering the key involvement of mitochondrial defects, the resulting altered metabolism in the progression of CLL, as well as the known effect of P53 on mitochondria, they examined the expression level of hsa-circ-0089762/mc-COX2 in relation to the P53 status in patients, and it was revealed that patients with P53 deletion compared to patients with P53 mutation have a significantly higher level of hsa-circ-0089762/mc-COX2. Similarly, data from in vitro analyses using the silencing of this mecciRNA by siRNA-circCOX2 in CLL cell lines including MEC-1 (*TP53*-mutant), JVM-3 (TP53-wild-type) as well as a cell line called CLL-1 isolated from patients indicated that the suppression of hsa-circ-0089762/mc-COX2 resulted in a decrease in ATP production and a lowered mitochondrial membrane capacity. Furthermore, their findings about apoptosis and cell proliferation revealed that the suppression of hsa-circ-0089762/mc-COX2 by affecting mitochondrial function inhibited cell proliferation and conversely induced cell apoptosis [29]. Likewise, Hong et al. reported a significant upregulation of the hsa-circ-0089762/mc-COX2 in tumor tissue compared to paracancerous tissue of patients with pancreatic cancer [56]. Other reported data on the expression of circRNAs in the parathyroid tissue of patients with sporadic parathyroid adenoma revealed a sex-specific increased expression level of hsa-circ-0089762 in affected males compared to affected females, and it was also bioinformatically shown that hsa-circ-0089762 exerts its functional role through sponging a set of miRNAs [57]. It was also found that the expression level of hsa-circ-0089762 is increased in cervical cancer [58]. Therefore, these

different data in various cancers, which are in line with or opposite to our results, can be indicative of a situation in which the altered expression of circ_0089762/mc-COX2 is derived by diverse and unknown specific pathological molecular interactions in different tumor tissues which are raised by cancer-specific mitochondrial-dependent tumorigenesis mechanisms. Regarding CRC, it can be assumed that the significant abnormal downregulation of this mitochondrial circRNA can lead to deviation in the metabolic program of tumor cells via playing a tumor suppressor role.

Besides, evidence from other studies on non-cancerous diseases has designated the aberrant expression of this mecciRNA. For instance, in line with our study, Zhao and colleagues found a reduced expression of SCAR in liver tissue as well as fibroblasts isolated from the liver tissues of patients with NASH, which clinically was also linked to its progression [30]. Mechanistically, the authors of this study revealed that lipid accumulation-mediated mitochondrial stress (switch of ATP production from oxidative phosphorylation cycle to glycolysis) and endoplasmic reticulum stress in liver fibroblasts opens mitochondrial permeability transition pore (MPTP) channel for releasing the mROS through silencing the SCAR by inhibiting the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), as a transcription activator of SCAR. The binding of SCAR/mc-COX2 to ATP5B prevents the interaction of CypD with mPTP, which subsequently results in the closing of the mPTP. This closure reduces mROS release and prevents fibroblast activation. This condition activates fibroblasts, leading to excessive production of inflammatory cytokines inducing metaflammation, as well as high production of collagen, both of which are involved in the development of NASH [30]. Clinically, the potential therapeutic utility of SCAR/mc-COX2 has been demonstrated by the reduction of meta-inflammatory responses in a mouse model of NASH. This was achieved through the mitochondrial-specific delivery of SCAR/mc-COX2 overexpression vectors using mitochondria-targeting nanoparticles (mito-NPs) [30].

Also, a decreased expression of the SCAR in the blood samples of patients with chronic obstructive pulmonary disease (COPD) and Alzheimer's disease [59, 60]. Wu and co-authors demonstrated that the treatment of human retinal vascular endothelial cells (hRMVECs) with high glucose significantly reduced SCAR expression and mtDNA copy number, followed by hRMVEC cell proliferation. On the other hand, increasing the expression of SCAR led to a decrease in the levels of ROS and ATP, an increase in the activity of superoxide dismutase and catalase enzymes, and finally, an increase in cell proliferation [32]. The data of research in cell and tissue models of the inflammatory disease of sepsis in mice showed a reduced expression of mouse mSCAR homolog and this abnormality leads to increased production of mROS, followed by increased polarization and production of M1 macrophages and systemic inflammation through the production of cytokines Tnfα, Nos2, Il1β and Il6 [61]. Unlike these disorders, previous works have shown an increased expression level of SCAR in the tissue of diabetic foot ulcer patients [62], plasma of patients with dilated cardiomyopathy (DCM), and blood samples of acute myocardial infarction (AMI) patients [31, 63]. These reports provide another layer of data regarding the molecular pathological significance of SCAR/mc-COX2 abnormality via mitochondria-dependent pathways in various human diseases.

Conclusion

In this study, for the first time, we provided the data on the downregulation of the expression of a new mecciRNA SCAR/mc-COX2 (hsa-circ-0089762) in CRC tumor tissue, which could be in line with its tumor suppressive role in this cancer. Physiopathologically, it was found that there was a significant correlation between its lower expression and higher pathological T stages in CRC tumors. Also, our data achieved a diagnostic biomarker value for SCAR/mc-COX2. Functionally, using bioinformatic data, we revealed that this mecciRNA mainly functions through sponging of some miRNAs such as hsa-miR-938 and hsa-miR-1249 to control several mRNAs, previously known in CRC tumorigenesis, in the form of specific gene expression regulatory ceRNA networks including hsa-circ-0089762/hsa-miR-938/FOXJ3, MGLL, FAM107A, FAM120AOS and hsa-circ-0089762/hsa-miR-1249/PLCG2. However, due to the limitations of this study, future studies are needed to investigate the ceRNA network associated with SCAR/mc-COX2. These functional studies could play an important role in revealing the role of SCAR/mc-COX2 in the tumorigenesis process of CRC and determining its potential as a biomarker for diagnostic and therapeutic purposes.

Abbreviations

AMI	Acute Myocardial Infarction
AUC	Area Under the Curve
BP	Biological Processes
ceRNA	Competing endogenous RNA
CLL	Chronic Lymphocytic Leukemia
COAD	Colon Adenocarcinoma
COPD	Chronic Obstructive Pulmonary Disease
CRC	Colorectal Cancer
DCM	Dilated CardioMyopathy
GEO	Gene Expression Omnibus
GEPIA	Gene Expression Profiling Interactive Analysis
GO	Gene Ontology
hRMVECs	Human Retinal Vascular Endothelial Cells
KEGG	Kyoto Encyclopedia of Genes and Genomes
mecciRNAs	Mitochondrial-encoded circular RNAs

MF	Molecular Function
miRNA	MicroRNAs
mito-NPs	Mitochondria-targeting NanoParticles
mPTP	Mitochondrial Permeability Transition Pore
mROS	Mitochondrial ROS
mtDNA	Mitochondrial DNA
mt-ncRNAs	Mitochondrial-encoded non-coding RNAs
NASH	Nonalcoholic Steatohepatitis
OXPHOS	Oxidative Phosphorylation
PPI	Protein-protein Interaction
PGC-1α	Peroxisome Proliferator-activated receptor Gamma Coactivator 1-alpha
qRT-PCR	Quantitative Real-Time PCR
RBP	RNA Binding Protein
READ	Rectum Adenocarcinoma
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SCAR	Steatohepatitis-associated CircRNA ATP5B Regulator
TCGA	The Cancer Genome Atlas
TME	Tumor Microenvironment

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Authors' contributions
Faezeh Mohamadhashem participated in laboratory education, coordinated the study, and revised the final manuscript. A Daraei devised the project, and the main conceptual, and revised the final manuscript. SA Mirzaei participated in laboratory education and revised the final manuscript. ST Nourbakhsh performed the experiments, analyzed the data, conducted the bioinformatic work, and drafted the manuscript. Fatemeh Mohamadhashem provided valuable medical information and assisted with the revision of the manuscript. M Naghizadeh conducted the bioinformatic work and performed the statistical analysis. AN Emami Razavi contributed to sample data collection and coordinated obtaining samples. Y Mansoori participated in intellectual discussions of the data.

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Data availability
Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and followed all applicable institutional and national guidelines and regulations for conducting research involving human participants. The experimental procedures were reviewed and approved by the Ethics Committee of Shahrekord University of Medical Sciences (Approval ID: IR.SKUMS.MED.REC.1402.018). Human tissue samples used in this study were obtained from the Iran National Tumor Bank, which operates under strict ethical and regulatory standards. Informed consent was obtained from all donors by the Iran National Tumor Bank prior to sample collection.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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