Overexpression of COX7RP promotes tumor growth and metastasis by inducing ROS production in hepatocellular carcinoma cells

Guihu Wang¹,², Branimir Popovic³,⁴, Junyan Tao³,⁴, An Jiang¹,²

¹National-Local Joint Engineering Research Center of Biodiagnostics and Biotherapy, Second Affiliated Hospital, Xi’an Jiaotong University, Xi’an, Shaanxi, People’s Republic of China; ²Key laboratory of Environment and Genes Related to Diseases, Xi’an Jiaotong University, Ministry of Education of China, Xi’an, Shaanxi, People’s Republic of China; ³The Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; ⁴The Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

Abstract: Cumulative evidence has indicated that mitochondrial respiration dysfunction plays important roles in tumorigenesis. However, the role of COX7RP, a critical regulator in the formation of mitochondrial respiratory supercomplex that has been suggested to be over-expressed in hepatocellular carcinoma (HCC) by our bioinformatic analysis of TCGA data, in tumor progression remains largely unclear. In this study, we found that COX7RP is frequently over-expressed in HCC mainly due to the down-regulation of miR-130a-3p and predicts poor prognosis of HCC patients. Functional experiments revealed that COX7RP promoted both growth and metastasis of HCC through induction of cell cycle progression and epithelial to mesenchymal transition (EMT), and suppression of cell apoptosis. Mechanistically, increased generation of reactive oxygen species (ROS) and subsequently activated nuclear transcription factor-κB (NF-κB) signaling was found to contribute to the promotion of HCC cell growth and metastasis by COX7RP. Collectively, COX7RP plays a critical oncogenic role in hepatocellular carcinogenesis, supporting COX7RP as a novel prognostic factor and therapeutic target in HCC.

Keywords: COX7A2L, reactive oxygen species, growth, metastasis, HCC

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, which is now the second leading cause of cancer-related death worldwide [1, 2]. Although great progress has been made during recent years in diagnostic techniques, and surgical and adjuvant systemic treatment, the prognosis continues to be poor for patients with HCC [3, 4]. Mitochondria are essential organelles critical for normal cell function such as cell metabolism and redox balance. For decades, cancer cells have been regarded as defective in mitochondrial respiration due to their hyperactivated glycolysis metabolism [5, 6]. Whereas, a growing body of evidence also has revealed the dependency of tumor cells on mitochondrial respiration and important roles played by abnormal mitochondrial respiration in tumorigenesis [7, 8]. Mitochondrial are not only the powerhouse for ATP production, but also the source of oncogenic metabolites such as α-KG and succinate, both of which play crucial roles in epigenetic regulation in cancer cells [9, 10]. In addition, mitochondria are also the main source of reactive oxygen species (ROS), which activates diverse signaling networks that drive tumor cell proliferation, survival and malignant progression [11, 12]. Thus, identification of novel potential molecular regulators involved in the dysfunction of mitochondrial respiration may provide insights into the biological basis of cancer development, as well as new potential diagnostic and therapeutic targets for treatment of this malignancy.
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Oxidative gene involved in the formation of mitochondrial respiratory supercomplex to facilitate efficient energy generation [13]. A recent study has shown that COX7RP is over-expressed in breast and endometrial cancer cells and promotes tumor cell growth both in vitro and in vivo [14]. However, the expression and biological effects of COX7RP in other cancer types are still largely unknown, especially in HCC.

In the present study, we systematically investigated the expression, biological effects and prognostic significance of COX7RP in HCC.

**Materials and methods**

**Cell culture**

Human hepatoma cell lines HLE, HLF, SNU-354, SNU-368 and SNU-739, and a normal hepatocyte HL-7702 cells were purchased from the Japanese Collection of Research Bioresources (Osaka, Japan). The cells were maintained in RPMI-1640 (Invitrogen, Grand Island, NY, USA) or DMEM (Gibco, Grand Island, NY, USA) culture medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA) in a humidified incubator containing 5% CO₂ at 37°C. Cell identities were authenticated by short tandem repeat profiling.

In addition, a total of 173 paired primary HCC tumor and adjacent non-tumor samples were collected during operation in the Second affiliated Hospital of Xi’an Jiaotong University in Xi’an, China. Written informed consents were obtained from each of the patients. The study was approved by the Ethics Committee of Xi’an Jiaotong University and carried out in accordance with the Declaration of Helsinki.

**Quantitative real-time PCR**

Quantitative Real-time PCR (qRT-PCR) was conducted with a 2 × SYBR Green qPCR Master Mix (S2014, USA Everbright Inc, Suzhou, China). Relative expressions of target genes were normalized to β-actin. The sequences of PCR primers used in this study are listed in Table S2.

**Western blot and immunohistochemistry (IHC) analysis**

Proteins from lysed HCC cell lines or tumor tissue samples were separated on SDS polyacrylamide gels and transferred onto the PVDF membrane. The membranes were then incubated with primary and appropriate secondary antibodies. The specific blots are detected by enhanced chemiluminescence assay (ECL; Amersham Pharmacia Biotech). Primary antibodies used in the study are listed in Table S3.

For IHC analysis, tissue sections were hydrated and treated with hot citrate buffer to unmask epitopes. After incubated with primary antibodies overnight at 4, immunohistochemical staining was applied with an IHC detection kit from MXB Biotechnology (Mxb, Fuzhou, China).

**Plasmids, transfection and RNA interference**

For over-expression of COX7RP, the coding sequence of COX7RP was amplified by PCR from SNU-739 cells and cloned into a pcDNA3.1(C) vector. For transiently knockdown of COX7RP, small interference RNA (siRNA) targeting COX7RP was purchased from Genepharm (Shanghai, China) and transfected into HCC cells using lipofectamine 2000 following to the manufacturer’s protocols. For generation of shRNA expression vector targeting COX7RP, a pSilencer™ 3.1-H1 puro vector was used. Stable knockdown or over-expression of COX7RP in HCC cells were derived from transient transfection followed by selection with G418 or puromycin.

**MTS cell viability and colony formation assays**

HCC cells were seeded into 96-well plates (020096, Xinyou Biotech, Hangzhou, China) at a density of 2000 cell/well. Then, 10 μl of MTS solution was added to HCC cells in each well to determine cell viability. Growth curve of HCC cells in each group was plotted with data (absorbance at 490 nm) collected from 0, 1, 2, 3 and 4 days. For colony formation assay, 1,000 cells were seeded into 6-well plate and cultured about two weeks. At last, the stained colonies were pictured and numbered for statistical analysis.

**Cell cycle and apoptosis assays**

Cell cycle was analyzed with 70% ethanol-PBS and propidium iodide (PI) solutions. Cell apoptosis was analyzed with a FITC-Annexin V and PI Apoptosis Kit (F6012, US Everbright Inc, Suzhou, China). A flow cytometry (Beckman, Fullerton, CA) was used for data collection and
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analysis. TUNEL assays were performed using an in-situ cell death kit from Roche Biochemicals according to the manufacturer’s protocols.

Wound-healing and transwell invasion assay

Cell migration was analyzed by wound-healing assay. In brief, three scratch wounds in each well of the six-well plates were made with a plastic pipette tip when HCC cells reached 90% confluence. Photos of wound-closure were taken at 0 h and 24 h, respectively.

For analysis of cell invasion, BD BioCoat matrigel Invasion Chamber (BD Biosciences) was used. A total of 2 x 10⁵ HCC cells were seeded into the upper chamber in culture medium without FBS and cultured for 48 h. Invaded cells stained with crystal violet were counted under a microscopy.

Subcutaneous xenograft mouse models

HCC cells stably expressing control (shCtrl) or shCOX7RP vector were injected subcutaneously into the right dorsal flank of BALB/c nude mice (four to five weeks of age, six mice per group). Tumor size was measured every week for five weeks using a Vernier caliper. The mice were sacrificed 5 weeks after cell injection. Tumors were carefully harvested and their weights were measured. All animal experimental procedures were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Xian Jiaotong University.

Tail vein metastatic assay

HCC cells stably expressing control (shCtrl) or shCOX7RP vectors were injected into the lateral tail veins of each BALB/c nude mice (four to five weeks of age, six mice per group). The mice were sacrificed 5 weeks after cell injection and their lungs were removed and prepared for hematoxylin and eosin (H&E) staining. Metastasis in the lungs were finally counted under a microscopy.

Statistical analysis

All the results were expressed as mean ± SD and analyzed with SPSS 19.0 software (IBM, Chicago, IL, USA). For comparing means between two groups, student’s t-test was used. For comparing the difference between multiple groups, One-way ANOVA was used. Overall and recurrence-free survival in relation to COX7RP expression were evaluated by Kaplan-Meier survival curve and log-rank test. Differences with P-value less than 0.05 was taken as statistical significance.

Results

COX7RP was up-regulated in HCC and associated with poor prognosis in patients with HCC

Our bioinformatic analysis based on the public mRNA expression data from the TCGA revealed that COX7RP was significantly up-regulated in HCC, especially in high-stages of HCC (Figure 1A and 1B). The mRNA expression level of COX7RP was further examined in 30-paired tumor and peritumor tissues from HCC patients by quantitative real-time PCR (qRT-PCR). As shown in Figure 1C, COX7RP expression was significantly higher in tumor tissues of HCC when compared with their adjacent normal tissues. In keeping with the increased COX7RP mRNA expression level in HCC, the protein expression of COX7RP was also significantly upregulated in HCC, as evidenced by immunohistochemical (IHC) staining in tumor and peritumor tissues from 173 HCC patients (Figure 1D). Quantitative RT-PCR and western blot analysis further revealed that COX7RP was up-regulated in five HCC cell lines when compared with an immortalized human HL-7702 hepatocyte (Figure 1E and 1F). In addition, we found that COX7RP expression was positively associated with higher clinical stage, larger tumor size and distant metastasis (Table S1). Kaplan-Meier survival analysis indicated that high expression of COX7RP predicted poorer overall survival (OS) and recurrence-free survival (RFS) for patients with HCC (Figure 1G and 1H). In line with our results, the online web portal UALCAN-based survival analysis [15] also showed that HCC patients with high expression level of COX7RP had clearly poorer overall survival than those with low COX7RP (Figure 1I). Taken together, COX7RP is significantly over-expressed in HCC and over-expression of COX7RP predicts poor prognosis in patients with HCC.

COX7RP knockdown suppressed HCC growth via inducing cell cycle arrest and apoptosis

The frequent up-regulation of COX7RP in HCC cell lines and tissues suggests that COX7RP may play a tumor-promotive role in HCC. To investigate the functional roles of COX7RP in...
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Figure 1. COX7RP was up-regulated in HCC and associated with poor prognosis in patients with HCC. A and B. Bioinformatics analysis for mRNA expression level of COX7RP in HCC based on the data from TCGA. C. qRT-PCR analysis of COX7RP expression in 30-paired tumor and peritumor tissues from HCC patients. (T, tumor; P, peritumor). D. IHC staining of COX7RP in 173-paired HCC tumor and peritumor tissues. Scale bar, 50 μm. E and F. qRT-PCR and western blot analysis of COX7RP expression in five HCC cell lines and one immortalized human HL-7702 hepatocyte. G and H. Kaplan-Meier curves for overall survival (OS) and recurrence-free survival (RFS) of HCC patients stratified by COX7RP expression level. I. Prognostic analysis of COX7RP in HCC patients using the online web portal UALCAN. *P<0.05.

HCC, COX7RP expression was knocked-down in SNU-368 and SNU-739 cells, which have relatively high COX7RP expression (shown in Figure 1E and 1F). Knockdown of COX7RP was confirmed by qRT-PCR and western blot analysis (Figure 2A and 2B). MTS cell viability and colony formation assays indicated that knockdown of COX7RP significantly reduced cell viability and colony formation in SNU-368 and SNU-739 cells (Figure 2C and 2D). Flow cytometry analysis revealed that knockdown of COX7RP induced cell cycle arrest at G1 phase in SNU-368 and SNU-739 cells (Figure 2E). In line with this, COX7RP knockdown reduced the expressions of the promoters of G1-S transition (CDK4 and cyclin D1), while enhanced the expressions of the gatekeepers of G1-S transition (p27 and p21) (Figure 2F). In addition, COX7RP knockdown induced cell apoptosis in SNU-368 and SNU-739 cells (Figure 2G), which was further confirmed by increased key cell apoptosis regulators (cleaved caspase-3 and caspase-7) (Figure 2H). These results collectively indicate that knockdown of COX7RP suppressed HCC growth mainly through inducing cell cycle arrest and apoptosis.
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Figure 2. COX7RP knockdown suppressed HCC growth via inducing cell cycle arrest and apoptosis. A and B. Knockdown of COX7RP in SNU-368 and SNU-739 cells was confirmed by qRT-PCR and western blot analysis. siCOX7RP, siRNA against COX7RP; siCtrl, control siRNA. C and D. The effect of COX7RP knockdown on cell viability and colony formation abilities in SNU-368 and SNU-739 cells with treatment as indicated. E. The effect of COX7RP knockdown on cell cycle distribution in SNU-368 and SNU-739 cells with treatment as indicated. F. The effect of COX7RP knockdown on the expression levels of cell cycle regulators were determined by western blot analysis in SNU-368 and SNU-739 cells with treatment as indicated. G. The effect of COX7RP knockdown on cell apoptosis in SNU-368 and SNU-739 cells with treatment as indicated. H. The effect of COX7RP knockdown on the expression levels of key cell apoptosis regulators were determined by western blot analysis in SNU-368 and SNU-739 cells with treatment as indicated. *P<0.05.
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COX7RP knockdown suppressed migration and invasion of HCC cells

We next investigated the effect of COX7RP on the migration and invasion abilities of HCC cells using in vitro wound healing and transwell invasion assays. As shown in Figure 3A and 3B, knockdown of COX7RP significantly suppressed cell migration and invasion abilities in both SNU-368 and SNU-739 cells. Meanwhile, qRT-PCR and western blot analysis showed that COX7RP knockdown significantly suppressed the epithelial-mesenchymal transition (EMT) through the down-regulation of mesenchymal regulators (N-cadherin and Vimentin) and up-regulation of epithelial regulators (E-cadherin and ZO-1) (Figure 3C and 3D). These findings indicate that knockdown of COX7RP suppressed migration and invasion of HCC cells mainly through inhibiting EMT.

COX7RP knockdown decreased HCC cell growth and metastasis in nude mice

To further investigate the effects of COX7RP on HCC growth and metastasis in vivo, shCtrl or shCOX7RP stably transfected SNU-368 cells (Figure S1A and S1B) were subcutaneously injected into nude mice. Tumors in shCOX7RP group grew significantly slower than those in shCtrl group (Figure 4A). In addition, tumor weight in the shCOX7RP group was much lighter than that in shCtrl group (Figure 4B). IHC analysis revealed significantly decreased COX7RP expression in xenograft tumor tissues from shCOX7RP group as compared with shCtrl group, indicating that the tumor growth suppressive effect was exerted by knockdown of COX7RP (Figure 4C). In addition, significantly fewer proliferating and more apoptotic cells were observed in xenograft tumor tissues from shCOX7RP group when compared with shCtrl group, as evidenced by Ki-67 and TUNEL staining assays (Figure 4D and 4E). In vivo tail vein metastatic assay further indicated that knockdown of COX7RP significantly decreased metastatic nodules formed in the lungs (Figure 4F).

Over-expression of COX7RP increased HCC cell growth and metastasis

To provide further support on the oncogenic roles of COX7RP in HCC, COX7RP was over-expressed in HLF and SNU-354 cells with relatively low COX7RP expression (shown in Figure 1E and 1F). Over-expression of COX7RP in HLF and SNU-354 cells (Figure S2A and S2B) markedly increased both cell viability and colony formation abilities of HCC cells (Figure 5A and 5B). As expected, COX7RP overexpression also induced G1/S cell cycle transition, but inhibited cell apoptosis in HLF and SNU-354 cells (Figure 5C and 5D). Moreover, COX7RP over-expression significantly enhanced migration and invasion abilities in HLF and SNU-354 cells (Figure 5E and 5F). Taken together, these results provide further support that COX7RP play a crucial oncogenic role in the promotion of HCC growth and metastasis.

Over-expression of COX7RP was mainly mediated by the down-regulation of miR-130a-3p

MicroRNAs (miRNAs) play important roles in carcinogenesis by regulating post-transcriptional gene expression. To identify the candidate microRNAs involved in the over-expression of COX7RP in HCC, an online microRNA Data Integration Portal (mirDIP)-based target prediction was applied. Among the top ten predicted miRNAs targeting COX7RP (Figure S3), miR-130-3p has been reported to be down-regulated in HCC [16] and several other types of cancer [17, 18]. We found that transfection of miR-130a-3p mimic significantly down-regulated COX7RP expression at both mRNA and protein levels in HCC cells (Figure 6A and 6B). To provide further support, the expression levels of miR-130a-3p were determined by qRT-PCR analysis in tumor tissues from 30 patients with HCC. As expected, miR-130a-3p level is negatively associated with the mRNA level of COX7RP (Figure 6C). Moreover, transfection of miR-130a-3p mimic also greatly attenuated COX7RP-promoted growth and metastasis in HCC cells (Figure 6D-G).

COX7RP promoted HCC growth and metastasis through activating ROS/NF-κB signaling

Mitochondrial have been well established as the main source of reactive oxygen species (ROS) in mammalian cells, which drive tumorigenesis through activation of multiple oncogenic signaling pathways such as AKT, NF-κB, and Hif-1α [11]. Accordingly, the level of ROS and its potential downstream oncogenic signaling pathways were evaluated in HCC cells with COX7RP knocked-down or over-expressed. As shown in Figure 7A, COX7RP knockdown...
Figure 3. COX7RP knockdown suppressed migration and invasion of HCC cells. A. Wound healing assay for determination of cell migration in SNU-368 and SNU-739 cells after transfection with siCOX7RP or siCtrl. Scale bar, 50 μm. B. Transwell Matrigel invasion assay for cell invasion ability in SNU-368 and SNU-739 cells after transfection with siCOX7RP or siCtrl. Scale bar, 20 μm. C and D. qRT-PCR and western blot analysis for expressions of key EMT regulators in SNU-368 and SNU-739 cells with treatment as indicated. *P<0.05.
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significantly decreased ROS levels in SNU-368 and SNU-739 cells, while forced expression of COX7RP in HLF and SNU-354 cells markedly increased ROS level. In addition, COX7RP knockdown suppressed the activation of AKT and NF-κB signaling, while did not notably affect the activation of Hif-1α, as evidenced by western blot analysis. In contrast, forced expression of COX7RP activated AKT and NF-κB (Figure 7B). In addition, NF-κB activation was markedly reversed by treatment with \( \text{H}_2\text{O}_2 \) or an ROS scavenger NAC in HCC cells, indicating that COX7RP over-expression activated ROS/NF-κB signaling in HCC cells (Figure 7C).

Given that COX7RP activated the oncogenic signaling of ROS/NF-κB, we hypothesized that the activation of ROS/NF-κB signaling may contribute to the promotion of HCC growth and metastasis by COX7RP. As shown in Figure 8A-D, \( \text{H}_2\text{O}_2 \) treatment significantly restored COX7RP knockdown suppressed HCC growth and metastasis, as determined by MTS cell viability, colony formation, wound healing migration and transwell invasion assays. In contrast, NAC treatment significantly attenuated COX7RP over-expression promoted HCC growth and metastasis. These results suggest that the activation of ROS/NF-κB signaling contributes the oncogenic property of COX7RP in HCC.

**Discussion**

In the present study, we have demonstrated that COX7RP, a critical factor involved in assembly of mitochondrial respiratory supercomplex, was frequently over-expressed in HCC mainly due to the down-regulation of miR-130a-
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A

B

C

D

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Figure 5. Over-expression of COX7RP increased HCC cell growth and metastasis. A and B. The effect of COX7RP over-expression on cell viability and colony formation abilities in HLF and SNU-354 cells after transfection with COX7RP expression vector (COX7RP) or empty vector (EV). C and D. The effect of COX7RP over-expression on cell cycle distribution and apoptosis in HLF and SNU-354 with treatment as indicated. E. The effect of COX7RP over-expression on cell migration ability in HLF and SNU-354 cells with treatment as indicated. Scale bar, 50 μm. F. The effect of COX7RP over-expression on cell invasion ability in HLF and SNU-354 cells with treatment as indicated. Scale bar, 20 μm. *P<0.05.
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A

B

C

D

E

F

Am J Cancer Res 2020;10(5):1366-1383
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Figure 6. Over-expression of COX7RP was mainly mediated by down-regulation of miR-130a-3p. A. qRT-PCR analysis for the expression of COX7RP in HCC cells transfected with synthetic miR-130a-3p. B. Western blot analysis for the expression of COX7RP in HCC cells transfected with synthetic miR-130a-3p. C. Correlation analysis between the expression levels of COX7RP and miR-130a-3p in tumor tissues from 30 HCC patients. D and E. MTS cell viability and colony formation assays in HCC cells treated as indicated. F. Wound-healing assay in HCC cells treated as indicated. Scale bar, 50 μm. G. Transwell Matrigel invasion assay in HCC cells treated as indicated. Scale bar, 20 μm. *P<0.05.
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Additionally, Kaplan-Meier analysis revealed that HCC patients with elevated COX7RP expression levels had significantly poorer survival than patients with low COX7RP expression levels. Consistently, a recent study also has demonstrated the overexpression COX7RP in breast cancer and its over-expression was associated with poor prognosis of breast cancer patients [14]. These observations suggest that dysfunction of mitochondrial respiratory supercomplex assemble plays a critical role in the progression of human cancers.

Elevated COX7RP expression in HCC cells suggested that COX7RP could act as an oncogene in the carcinogenesis and progression of HCC. In this connection, the biological functions of COX7RP were explored by a series of in vitro and in vivo functional experiments in HCC cells. Knockdown of COX7RP significantly suppressed HCC growth in SNU-368 and SNU-739 cells, while COX7RP over-expression significantly accelerated HCC cell growth in HLF and SNU-354 cells. Moreover, we found that COX7RP promoted HCC cell growth through induction of G1-S cell cycle transition as well as suppression of cell apoptosis. In line with our results from HCC cells, over-expression of COX7RP in breast cancer also has been shown to promote tumor cell proliferation and growth [14].

Metastasis is the primary cause of cancer mortality. Accordingly, the functional roles of COX7RP in the migration and invasion abilities of HCC cells were also explored. Knockdown of COX7RP significantly inhibited the migration and invasion abilities of HCC cell in SNU-368 and SNU-739 cells, while COX7RP over-expression markedly increased the migration and invasion abilities in HLF and SNU-354 cells. Consistently, several lines of evidence also have indicated that increased mitochondrial respiration and increased ATP production are essential for metastasis of cancer cells [19-21]. These observations collectively suggest that mitochondrial respiration is crucial for the maintenance of metastasis phenotype for tumor cells. Additionally, our results further indicated that the metastasis promoting role of COX7RP was mainly mediated by the activation of epithelial-mesenchymal transition (EMT).
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A

SNU-354

- siCtrl
- siCtrl+H2O2
- siCOX7RP
- siCOX7RP+H2O2

SNU-739

- EV
- EV+NAC
- COX7RP
- COX7RP+NAC

B

SNU-354

Colony Number

- siCtrl
- siCtrl+H2O2
- siCOX7RP
- siCOX7RP+H2O2

SNU-739

Colony Number

- EV
- EV+NAC
- COX7RP
- COX7RP+NAC

C

SNU-354

Wound closure (%)

- siCtrl
- siCtrl+H2O2
- siCOX7RP
- siCOX7RP+H2O2

SNU-739

Wound closure (%)

- EV
- EV+NAC
- COX7RP
- COX7RP+NAC
Figure 8. COX7RP promoted HCC growth and metastasis through activation of ROS/NF-κB signaling. A. MTS cell viability assay in HCC cells treated with 80 μM H$_2$O$_2$ or 25 mM NAC for 12 h, respectively. B. Colony formation assay in HCC cells with treatment as indicated. C. Wound healing assay for cell migration ability in HCC cells with treatment as indicated. Scale bar, 50 μm. D. Transwell Matrigel invasion assay for invasion abilities in HCC cells with treatment as indicated. Scale bar, 20 μm. *P<0.05.
MiR-130a-3p has been reported as a frequently down-regulated tumor suppressor in HCC and several other types of cancers [16-18]. Consistently, our present study also indicated a significant down-regulation of miR-130a-3p in HCC cells. Moreover, we found that down-regulation of miR-130a-3p was involved in the over-expression and tumor promotive effects of COX7RP in HCC cells. However, we still cannot rule out the possibility that some other genetic or epigenetic alterations could also contribute to the over-expression of COX7RP in HCC cells, which still needs further evaluation.

Mitochondria are the main source of reactive oxygen species (ROS), which is usually increased along with the activation of mitochondrial respiration [22]. In addition, several previously studies have demonstrated that the supercomplex formation could generate mitochondrial ROS during oxidative phosphorylation [23]. In this study, we found that COX7RP over-expression increased ROS production and thus the activation of NF-κB signaling in HCC cells. Moreover, we found that the activated ROS/NF-κB signaling was involved in the promotion of HCC cell growth and metastasis by COX7RP.

In summary, COX7RP is frequently over-expressed in HCC mainly due to the down-regulation of miR-130a-3p. COX7RP plays a critical oncogenic role in the promotion of HCC growth and metastasis, suggesting that COX7RP could serve as an important prognostic factor and therapeutic target in HCC.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. An Jiang, National-Local Joint Engineering Research Center of Biodiagnostics and Biotherapy, Second Affiliated Hospital, Xi’an Jiaotong University, No. 157, West 5th Road, Xi’an 710004, Shaanxi, People’s Republic of China. Tel: +86-029-87679684; E-mail: doctorj@126.com

References

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**Table S1.** Correlation of COX7RP expression with clinicopathologic features in 173 HCC patients

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Abbreviations: HBsAg, hepatitis B virus surface antigen; AFP, alpha-fetoprotein; PVTT, portal vein tumor thrombosis; TNM, tumor-nodes-metastases; TACE, transcatheter arterial chemoembolization.
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**Table S2.** Sequence of primers for qRT-PCR analysis

<table>
<thead>
<tr>
<th>Primers used in q-PCR analysis</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX7RP</td>
<td>TTCACGCAGAAGTTGGCAGG</td>
<td>GAGGTCAGTTTAGTGGTGTTGG</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>AAAGGCCATTTCTAAAAACCT</td>
<td>TGGTTTCTCATCCAGAGGCT</td>
</tr>
<tr>
<td>ZO-1</td>
<td>CGACCAGATCCTCAGGGTG</td>
<td>TCCATAGGGAGATCCTCCTCA</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>AGCTCCATTCCGACCTAGACA</td>
<td>CAGCCTGAGACAGAGAGT</td>
</tr>
<tr>
<td>Vimentin</td>
<td>GACGCCATCAACCAGGTT</td>
<td>CTTTGTGTTGATTTCCTCA</td>
</tr>
<tr>
<td>miR-130a-3p</td>
<td>GGGGGTGCAATGATGAATG</td>
<td>GTTCTGCTGAGGGAGTCG</td>
</tr>
<tr>
<td>U6</td>
<td>GCCGACGACCATATAATGCA</td>
<td>TCGCTTTGCGATCCG</td>
</tr>
<tr>
<td>β-actin</td>
<td>TCGCCTTTGCGATCCG</td>
<td>ATGATCTGTTGATCATCCTC</td>
</tr>
</tbody>
</table>

**Table S3.** Primary antibodies used in the study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Company (Cat. No.)</th>
<th>Working dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX7RP</td>
<td>abcam (ab66014)</td>
<td>WB: 1/1000; IHC: 1/200</td>
</tr>
<tr>
<td>CDK4</td>
<td>Proteintech (11026-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>cyclin D1</td>
<td>Proteintech (60186-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>p27</td>
<td>Proteintech (25614-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>p21</td>
<td>Proteintech (10355-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>abcam (ab1416)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>ZO-1</td>
<td>abcam (ab190085)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>abcam (ab98952)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>Vimentin</td>
<td>abcam (ab8978)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Proteintech (27309-1-AP)</td>
<td>IHC: 1/300</td>
</tr>
<tr>
<td>Akt</td>
<td>Proteintech (10176-2-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>p-Akt</td>
<td>Proteintech (66444-1-IG)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>p65</td>
<td>Proteintech (10745-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
<tr>
<td>p-p65</td>
<td>Abcam (b86299)</td>
<td>WB: 1/800</td>
</tr>
<tr>
<td>Hif1α</td>
<td>Abcam (ab92498)</td>
<td>WB: 1/600</td>
</tr>
<tr>
<td>β-actin</td>
<td>Proteintech (20536-1-AP)</td>
<td>WB: 1/1000</td>
</tr>
</tbody>
</table>
COX7RP promotes HCC growth and metastasis

**Figure S1.** A and B. Stable knockdown of COX7RP in SNU-368 cells was determined by RT-PCR and Western blot analysis (shCOX7RP, shRNA expression vector against COX7RP; shCtrl, control shRNA).

**Figure S2.** A and B. Transiently forced expression of COX7RP in HLF and SNU-354 cells was determined by RT-PCR and Western blot analysis. COX7RP, expression vector encoding COX7RP; EV, empty vector.

**Figure S3.** Top ten predicted miRNAs targeting COX7RP.