ATO /miRNA-885-5p/MTPN axis induced reversal of drug-resistance in cholangiocarcinoma

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Research

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Abstract

Backgroud:

In recent years, the incidence of cholangiocarcinoma (CCA) has increased, and it has become the second most malignant tumor of the hepatobiliary system. Chemotherapy has become the main treatment for cholangiocarcinoma due to its difficulty in diagnosis and rapid progress. Primary drug resistance is the main reason for the poor efficacy of chemotherapeutic drugs.

Methods

Western blot and quantitative real-time PCR assays were used to detect the expression levels of myotrophin (MTPN) and microRNA-885-5p(miR-885-5p) in CCA tissues and cells; Cell viabilities treated with arsenic trioxide (ATO), 5-fluorouracil (5-Fu) and cisplatin (CDDP) were analyzed by a CCK-8 kit. Luciferase reporter assay detected the relationship between miR-885-5p and MTPN. Kaplan-Meier analysis showed survival time curve.

Results

We found that ATO can reduce the resistance of CCA cells to 5-Fu and CDDP and promote the killing effect of 5-Fu and CDDP. Low-dose ATO played an anti-drug-resistance role through up-regulating the expression of miR-885-5p. Combined with sequencing results and database prediction, we found that MTPN was a direct target gene of miR-885-5p. The sensitivity of CCA cells to 5-Fu and CDDP was increased after MTPN was knocked out. After MTPN knockout, the sensitivity of cholangiocarcinoma cells to 5-FU and CDDP was increased. ATO can reverse chemotherapy resistance induced by overexpression of MTPN.

Conclusions

Our study suggests that the ATO/miR-885-5p/MTPN axis is a potential therapeutic strategy for improving the sensitivity of CCA to chemotherapy.

Background

Cholangiocarcinoma is a malignant neoplasm originating from bile duct epithelium. It is poorly differentiated and highly invasive. The incidence of cholangiocarcinoma has been increasing year by year in the world, especially in Thailand and Italy. Cholangiocarcinoma has become the second most common hepatobiliary tumor [1–2]. Owing to its rapid progression and metastasis, only 15% of early patients have access to surgery and the recurrence rate is extremely high [3–4]. Radiotherapy and chemotherapy are the main treatment methods for most patients with cholangiocarcinoma. Cisplatin, 5-

fluorouracil and gemcitabine are commonly used in clinical combined chemotherapy. Different from other tumors, many patients are insensitive to drugs and show drug resistance from the beginning of treatment [5]. Therefore, it is advisable to overcome the primary drug resistance of cholangiocarcinoma and improve its sensitivity to classical chemotherapeutic drugs.

In decades, many studies have found that ATO can reverse drug resistance in a variety of tumors. Firstly, ATO selectively inhibits the efflux pump GST-π and regulates the expression of Topo-II and Bcl-2 to enhance the killing effect of Adriamycin(ADM) on leukemia cells[6]. Similarly, ATO can reduce the resistance of gastric cancer drug-resistant cell line SGC7901/ADM to ADM [7]. Compared with etoposide alone, non-toxic dose of ATO combined with etoposide significantly enhanced apoptosis in Ewing sarcoma cells [8]. In addition, arsenic can bind to p62 to induce autophagy, followed by reduction of drug resistance to gefitinib in non-small cell lung cancer (NSCLC) [9]. ATO functions not only in reducing chemotherapy tolerance, but also synergistically in promoting radiotherapy effect. In radiotherapy of neuroblastoma or metastatic paraganglioma/pheochromocytoma, ATO participates in reducing the dose of radiotherapy, as a sensitizer [10]. As we all know, in the treatment of hematological diseases and various solid tumors, ATO plays an anti-cancer role by increasing cell reactive oxygen (ROS), reducing telomerase activity, inducing cell apoptosis, blocking cell cycle arrest and proliferation. The transformation of arsenide is carried out in the liver, and the metabolites of arsenic, such as trivalent arsenic and pentavalent arsenic, are excreted into the bile duct. Hence, the liver and bile duct are the first places where arsenide, represented by ATO, plays its role [11–12]. Generally speaking, ATO can improve the sensitivity of cancer to radiotherapy and chemotherapy through many ways, which brings new hope for overcoming drug resistance of CCA.

MicroRNAs (miRNAs) play an important role in the occurrence and development of tumors through regulating post-transcriptional gene expression. miRNAs were involved in the influence of biological behaviors of the carcinomas caused by ATO. It has been found that ATO suppresses cell growth and migration via inhibition of miR-27a in breast cancer cells [13]. In addition, ATO inhibits CXCR4-mediated metastasis by interfering miR-520 h/PP2A/NF-κB signaling in cervical cancer [14]. Our previous study revealed that miRNA-217 was involved in the lncRNA-RMRP-induced proliferation, invasiveness and metastasis in CCA [15]. Except miRNA-217, expression disorder of a series of microRNAs occurred in CCA. Among them, dysregulation of miR-885-5p was the most obvious. It is reported that expression level of miR-885-5p is low in liver cancer cells and tissues, and it mediates Waborg effect by targeting hexokinase 2 [16]. Additionally, the overexpression of miR-885-5p in liver cancer cells can also target astrocyte elevation gene 1 to significantly inhibit cell migration, invasion, proliferation, angiogenesis and EMT [17]. Therefore, our present study aimed to investigate the relationship between ATO and miR-885-5p and their roles in primary drug resistance in CCA.

Materials And Methods

Clinical samples

Cholangiocarcinoma and normal t tissues (n = 35) were obtained by surgical operations from the Second Affiliated Hospital of Nanjing Medical University from 2014 to 2019. This study was approved by the Second Affiliated Hospital of Nanjing Medical University Review Board. All patients provided informed consent. All tissues were taken and rapidly placed in liquid nitrogen. All clinical experiments were approved by the research ethics committee of Nanjing Medical University (Nanjing, China).

Cell Culture and Experiment Reagents

Human CCA cell lines (RBE and HCCC-9810) and Bile duct epithelial cells HIBEpiC were obtained from Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (ICBC, Shanghai, China) and were cultured in RMPI1640 supplemented with 10% FBS, 100 μg/mL streptomycin, and 100 U/mL penicillin in a humidified atmosphere conditions containing 5% CO_2 at 37 °C. Arsenic trioxide (ATO, ATO, > 99.0% purity) and 5-Fluorouracil (5-Fu, C4H3FN2O2,> 99.0% purity) and Cisplatin (CDDP, Cl2H6N2Pt, > 99.0% purity) were purchased from Sigma-Aldrach (Shanghai, China).

Drug treatment and calculation of the 50% inhibitory dose (IC_{50})

The cells were treated with 5-Fu in concentration gradient (1 μM,100 μM, 200 μM, 500 μM,1000 μM) and ATO(1 μM,2 μM,5 μM,10 μM,20 μM,40 μM) and CDDP (1 μM,20 μM, 40 μM, 80 μM,160 μM).Cell viabilities were determined via using a CCK-8 kit (Promega, Madison, WI, USA). The IC_{50}s were calculated via a graphpad 7.0 software. The inhibition ratio was calculated with a non- linear regression. Each concentration is set in three parallels. To adapt to the calculation mode “log (inhibitor) vs. response (three parameters)”, OD value need to be converted to a logarithmic value. Graph-pad will draw the s-curve based on the input value and calculate the IC50 and p values.

Cell transfection

The cells were transfected with miR-885-5p mimic and inhibitor according to the manufacturer’s instructions (RiBoBio, GuangZhou, China). Three individual MTPN small interfering RNA, scrambled negative control (NC) siRNAs, pc-MTPN and pcDNA3.1 (+) vector were purchased and transfected into cells using Lipofectamine 3000 (ThermoFisher Scientific, Waltham, MA, USA).

RNA isolation and qPCR assays

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The levels of miR-885-5p and MTPN were measured in the CCA cell lines and sanmples obtained from hospital as described previously. The primers sequences were listed in TableS1.

Western blot assay

The protein (25 μg) were extracted from the transfected cells and quantified using a 15% polyacrylamide gradient SDS gel. We transfer it to polyvinylidene fluoride membranes (0.22μM, Millipore). Quantification

was undertaken using a density assay using ECL chromogenic substrate. A GAPDH Ab (Sigma) was used as a control to correct for differences. Anti-MTPN was purchased from Abcam. Image J, a software for densitometric analysis, were used to measure the protein bands on the blots.

Luciferase reporter assay

The myotrophin (MTPN) 3'-untranslated region (3'UTR) containing the wild type or mutated miR-885-5p binding sequences were synthesized by Genescript (Nanjing, China). We clone wild type or mutated 3'UTR into the pmirGLO luciferase reporter vector separately (Promega, Madison, WI, USA). The wild type/mutant MTPN luciferase reporter vector and miR-885-5p mimic/control were co-transfected into cells. Luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). Results were expressed as the firefly luciferase activity with Renilla luciferase activity as normalization.

Statistical analysis

Data analysis used SPSS22.0 (SPSS, Chicago, IL, USA). A unpaired *t* test was used to compare miR-885-5p expression in CCA tissue and matched normal tissue. Kaplan-Meier method was used to evaluate survival curves, and differences in survival distributions were assessed by the log-rank test. To compare classification data, chi-squared test was used. According to the level of miR-885-5p/MTPN expression, all patients were divided into miR-885-5p/MTPN high group and miR-885-5p/MTPN low group (demarcated by the median of miR-885-5p/MTPN expression). A $p < 0.05$ was considered statistically significant.

Results

Sensitization effect of ATO in cholangiocarcinoma cells

We treated cholangiocarcinoma cells RBE and HCCC-9810 with ATO according to the concentration gradient, and observed the effect of ATO on the activity of RBE and HCCC-9810 cells. In RBE, when the concentration of ATO was 2 μM, the cell viability was not affected evidently. The results in HCCC-9810 were similar (Fig. 1A). As a result, 2 μM was supposed to be the maximum non-toxic concentration of ATO on cholangiocarcinoma cells. We found that 2 μM ATO had no inhibitory effect on the activity of normal bile duct epithelial cell (HIBEpic), RBE and HCCC-9810 compared with the control group without ATO (Fig. 1B). Then, RBE and HCCC-9810 were treated with 2 μM ATO and 5-FU according to the concentration gradient. IC50 in RBE and HCCC-9810 decreased significantly, compared with 5-Fu treated alone. Similarly, the IC_{50} of the group treated with ATO combined with CDDP (0-160 μM) was significantly lower than that treated with CDDP alone (Fig. 1C). These results confirmed that low-dose ATO can significantly enhance the effect of common chemotherapy drugs for cholangiocarcinoma, such as 5-Fu, CDDP.

Effect of miR-885-5p on chemosensitivity of cholangiocarcinoma cell lines

Based on our previous study [20], thousands of microRNAs were expressed differently, and these microRNAs were considered to be "useful microRNAs" that affect the occurrence and development of CCA (Fig. S1A). Six differently expressed microRNAs were selected for PCR verification, two were up-regulated and four were down-regulated (Fig. S1B). We detected the expression of six miRNAs in HIBEpic, RBE and HCCC-9810. We found that there was distinct difference between the expression level of miR-885-5p in HIBEpic, RBE and HCCC-9810. Meanwhile, compared with HIBEpic, the expression level of miR-196a-5p was significantly higher. However, the expression level of the other four microRNAs was not significantly different (Fig. 2A). Consistent with the results in cells, the expression level of miR-885-5p in 35 CCA tissues was lower than that in normal tissues, while miR-196a-5p was opposite (Fig. 2B). Furthermore, the expression of miR-885-5p and miR-196a-5p of samples in The Cancer Genome Atlas (TCGA) supported our findings. We can speculate that miR-885-5p plays an anti-cancer role in CCA, while miR-196a-5p is an oncogene. To investigate the effect of miR-885-5p and miR-196a-5p on the chemoresistance of cholangiocarcinoma, we successfully transfected miRNA-885-5p mimic in RBE and HCCC-9810. After 24 hours of transfection, the two cells were treated with 5-Fu or CDDP according to the concentration gradient, the inhibiting effect on cell viability of the experimental group overexpressing miR-885-5p was significantly lower than that of the control group (Fig. 2C). However, after transfection of miR-196a-5p inhibitor, the inhibiting effect on cell viability of the experimental group was not significantly reduced, whether treated with 5-Fu or CDDP (Fig. S2). Consequently, we believe that miRNA-885-5p is a potential microRNA to improve the chemosensitivity of CCA.

ATO enhanced the chemosensitivity of cholangiocarcinoma cells through miR-885-5p

Due to the significant enhancement of chemosensitivity of ATO to cholangiocarcinoma cells and the particularity of miR-885-5p in the effect of chemotherapy, we speculated whether the enhancement of chemosensitivity by ATO was related to miR-885-5p. After treating RBE and HCCC-9810 with ATO (0–2 μM), we detected the expression of miR-885-5p. It was found that with the increase of ATO concentration, the expression level of miR-885-5p increased gradually, and it was the highest under 2 μM ATO (Fig. 3A). In order to further confirm the conjecture, we carried out rescue experiments. The rescue experiment also showed that ATO could reverse the reduced sensitivity of 5-Fu and CDDP in RBE and HCCC-9810 caused by miR-885-5p knock down (Fig. 3B). Therefore, the above results prove that ATO can promote chemosensitivity by inducing the expression of miR-885-5p.

MTPN was a target gene of miR-885-5p in CCA

According to the sequencing results, of the 16968 detected genes, each of them may be a target gene for one or even several microRNAs. These genes were shown by Heatmap (Fig. 4A). We found that 382 genes were differentially expressed. The differential multiple of 9 genes were greater than 5, 5 of which were up-regulated genes and 4 of which were down-regulated genes (Fig. S3). In order to identify the target gene of miRNA-885-5p, we predicted it in database starBase 3.0 and Target Scan 7.2. There were 155 target genes of miR-885-5p in starBase and 3082 in Targetscan. MTPN was the only one gene as the target gene of miRNA-885-5p, based on the predicted results and the results of second generation sequencing

(Fig. 4B). MTPN was first found in hypertrophic myocardium of mice and its role in promoting myocardial hypertrophy was also discovered [18]. However, in cancer, what role MTPN plays is poorly understood. To verify the possible effect of MTPN in cholangiocarcinoma, first of all, we found that the expression of MTPN in RBE and HCCC-9810 were significantly higher than that in HIBEpic (Fig. 4C). Then we carried out PCR experiments in 35 cholangiocarcinoma tissues and found that compared with the adjacent tissues, the expression of MTPN in 28 tissues were significantly higher. Besides, our findings concurred with the analysis of TCGA samples. (Fig. 4D). Additionally, the results of 36 specimens in starBase 3.0 database showed that MTPN expression was negatively correlated with miRNA-885-5p (Fig. 4E). It follows that there was a strong possibility that MTPN may involve in the multidrug resistance of CCA.

ATO regulated the expression of MTPN through miR-885-5p

As a result of the high expression of MTPN both in cholangiocarcinoma cells and tissues and the prediction results of database and second-generation sequencing, we used luciferase reporter gene experiment to confirmed the relationship between miRNA-885-5p and MTPN. First, the 2978–2985 base fragment of MTPN may be the binding region of miR-885-5p according to the prediction results. We constructed luciferase plasmids containing wild-type MTPN and mutant MTPN 3'UTR respectively. Then, miR-885-5p mimic or control was co-transfected with plasmid. The results showed that miR-885-5p only combined with 3'UTR of wild-type MTPN, and significantly down regulated the expression of luciferase gene in sea pansy where the 3'UTR sequence of MTPN was located (Fig. 5A). Because miR-885-5p can directly bind to MTPN, we observed that MTPN both in RNA and protein in the cholangiocarcinoma cells lines of which miR-885-5p was overexpressed was significantly down-regulated (Fig. 5B).Moreover, in RBE and HCCC-9810 treated with ATO, MTPN decreased both in RNA and protein, compared with the control group (Fig. 5C). We conducted rescue experiments and found that ATO could block the increase of MTPN caused by miR-885-5p knockdown in RBE and HCCC-9810 (Fig. 5D). Our findings showed that MTPN was a novel direct target gene of ATO-mediated miR-885-5p in cholangiocarcinoma.

ATO/miR-885-5p/MTPN axis improved the anti-drug-resistance of cholangiocarcinoma cells

In order to explore whether MTPN affects the drug resistance of CCA cells, we successfully know-downed MTPN in RBE and HCCC-9810, and then added 5-Fu to the cells according to the concentration gradient. Compared with the control group, the IC50 of the experimental group was markedly lower, similarly, IC50 of CDDP in RBE and HCCC-9810 decreased significantly after MTPN knock-out (Fig. 6A). The rescue experiment showed that IC50 of 5-Fu and CDDP in RBE and HCCC-9810 induced by MTPN overexpression was decreased by ATO treatment (Fig. 6B). Above results indicated that ATO can reverse the primary chemotherapy resistance of cholangiocarcinoma by regulating MTPN.

Validation of prognostic values of miR-885-5p and MTPN in CCA

To explore the relationship between miR-885-5p/MTPN and clinical characteristics in CCA, we divided 35 patients into “low miR-885-5p expression/high MTPN expression”,“high miR-885-5p expression/high MTPN expression or low miR-885-5p expression/low MTPN expression” and “high miR-885-5p

expression/low MTPN expression" groups. A Kaplan-Meier analysis showed that the patients in the"low miR-885-5p expression/high MTPN expression" group exhibited worse survival than those in the "high miR-885-5p expression/low MTPN expression" group. (Fig. 7). Apparently, miR-885-5p and MTPN could serve as biomarkers in the field of CCA. Collectively, these results indicated that the upregulation of miR-885-5p induced by ATO treatment may contribute to the decrease of MTPN and thereby lead to anti-drug resistance to 5-Fu and CDDP.

Discussion

ATO, as essential component of a traditional Chinese drug, its cytotoxic effects was proved by lots of researches in vitro and vivo. Kim et al found that ATO(1-100 μM) inhibited the growth of CC-t6 cells more effectively than CDDP or ADM in most time points of 24–72 hours except 1 μM ATO treatment. Apart from this, high-dose ATO (5 mg/kg), low-dose ATO (1 mg / kg) and saline were used as the control group for local injection of subcutaneous transplanted tumor [19]. For the reason that we put forward 2 μM was a non-toxic dose of ATO. When RBE and HCCC-9810 was treated with 2 μM ATO, the cell death was attributed to 5-Fu or CDDP and irrelevant to ATO. The role of 2 μM ATO is to improve the sensitivity of cells to 5-Fu or CDDP. Furthermore, because of its non-toxic to cells, ATO had great potential to be used in clinical treatment of CCA.In addition, in pancreatic cancer cells, the ATO/miR-330-5p/Skp2 axis inhibits the proliferation, invasion and metastasis of cancer cells and promotes cell apoptosis [20]. Similarly, ATO reduces the invasive ability of chondrosarcoma by up-regulating the expression of microRNA-125b-induced mesenchymal epithelial transformation (MET) through demethylation [21]. It follows that miRNAs were crucial to ATO-induced inhibition of the biological behavior of various malignant tumors.

The microRNA expression profile of CCA reveals the importance of a large number of microRNAs in the occurrence and development of cholangiocarcinoma. For example, miR-212 targeting FOXA1 inhibits the proliferation and invasion of CCA cells [22]. MiR-191 promotes the proliferation, migration and metastasis of CCA cells in vivo and in vitro through TET1-p53 pathway, and plays the role of tumor promoter [23]. Although we found that inhibiting the expression of miRNA-196a-5p did not improve the sensitivity of cells to 5-Fu, we believe that the high expression of miRNA-196a-5p in cancer tissues may affect CCA in other ways. It is worth noticing that miRNA-885-5p, as a tumor suppressor, had a low expression in CCA cells and tissues. ATO can enhance the level of miRNA-885-5p as a stimulator to play an anti-drug resistance role. Borgmastars et al revealed the tumor suppressing effect of miRNA-885-5p and affirmed it could act as a potential prognostic biomarker from 15 miRNAs in pancreatic cancer [24]. However, some studies have shown that increased levels of miRNA-885-5p in tissues or body fluids can be used as diagnostic markers for tumors and short survival period. [25–26]. Currently, studies involved in target genes of miRNA-885-5p are not enough. In osteosarcoma, down-regulation of β-catenin expression by miRNA-885-5p inhibits cell proliferation [27]. MiRNA-885-5p targets cyclin-dependent kinase (CDK2) and microchromosome maintenance protein (MCM5), activating p53 to interfere with cell cycle and growth [28]. These results suggested that we can further expand the population sample to improve persuasiveness of our findings. Following our findings in CCA cells, we could further verify whether up-

regulation of miRNA-885-5p can show anti-drug ability in mouse models. Moreover, the effect of miRNA-885-5p in cell proliferation, invasion and metastasis were worth our continue study.

Our experiments revealed the role of ATO in reversing drug resistance to CCA chemotherapy, and for the first time demonstrated the significance of MTPN as a direct target of microRNA-885-5p in CCA. MTPN was first discovered in mammalian hypertrophic myocardium and mouse brain. It was subsequently confirmed that it was stable in all tissues and highly homologous among mammals [18, 29–30]. At present, the studies about MTPN are very few. Most of them were just focus on two fields, one of them was that MTPN promotes cardiac hypertrophy-induced heart failure [31], and the other one is its participation in insulin secretion regulated by the specific expression of microRNA-375 in pancreas [32–33]. Muniz Lino MA et al found the abundance of MTPN proteins was increased in triple-negative breast cancer (TNBC) tissues [34]. According to the analysis of GO and KEGG in previous studies, the differentially expressed genes involve multiple intracellular and extracellular functions. As a result of our research purpose, the filtered MTPN has strong pertinence. Knockdown of MTPN promoted the sensitivity of CCA cells to 5-Fu and CDDP. Kaplan-Meier survival analysis suggested that high miRNA-885-5p and low MTPN may act as a signal of better prognosis. We have only covered a bit of the biological functions of MTPN. It has been found that MTPN and p65 are co-transported to the nucleus of cardiac myocytes based on the structure of MTPN hairpin, which induces protein synthesis and myocardial growth [35]. Owing to the mechanism of MTPN leading to cell drug resistance is still not clear, it may be the next research direction in CCA.

Conclusion

In conclusion, our study demonstrated that ATO can enhance the chemosensitivity of CCA cells to 5-Fu and CDDP. The combination of ATO and miR-885-5p mimics may be a potential therapeutic strategy for CCA. ATO/miR-885-5p/MTPN axis may be a therapeutic approach to overcome resistance to CCA chemotherapy.

Abbreviations

5-Fu, 5-Fluorouracil; ATO, arsenic trioxide; CCA, Cholangiocarcinoma; CDDP, Cisplatin; miRNAs, microRNAs; MTPN, myotrophin; ROS, reactive oxygen species

Declarations

Ethics approval and consent to participate

This study has been conducted in accordance with ethical standards and according to the Declaration of Helsinki and the national and international guidelines, and has been approved by the authors' institutional review board. The study protocol was approved by the Medical Ethics Committee of Nanjing Medical University.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors have no conflict of interest.

Authors' contributions

YW, WZ, LC, WC and SX contributed equally to this work.

LM, QL, QJ conceived the research. YW and QL drafted the manuscript. LM, QL, QJ reviewed and revised it critically. YW, WZ, LC, WC and SX performed the experiments. LT and YY collected the clinical samples and analyzed the data. All the authors have read and approved the final version of the manuscript.

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References

1. Banales JM, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, et al. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). Nature reviews Gastroenterology & hepatology. 2016;13(5):261-80.
2. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology. 2013;145(6):1215-29.
3. Lustri AM, Di Matteo S, Fraveto A, Costantini D, Cantafora A, Napoletano C, et al. TGF-beta signaling is an effective target to impair survival and induce apoptosis of human cholangiocarcinoma cells: A study on human primary cell cultures. PloS one. 2017;12(9):e0183932.
4. Klempnauer J, Ridder GJ, Werner M, Weimann A, Pichlmayr R. What constitutes long-term survival after surgery for hilar cholangiocarcinoma? Cancer. 1997;79(1):26-34.
5. Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet (London, England). 2014;383(9935):2168-79.
6. Zhao D, Jiang Y, Dong X, Liu Z, Qu B, Zhang Y, et al. Arsenic trioxide reduces drug resistance to adriamycin in leukemic K562/A02 cells via multiple mechanisms. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2011;65(5):354-8.
7. Zhao YY, Yu L, Liu BL, He XJ, Zhang BY. Downregulation of P-gp, Ras and p-ERK1/2 contributes to the arsenic trioxide-induced reduction in drug resistance towards doxorubicin in gastric cancer cell lines. Molecular medicine reports 2015; 12(5): 7335-43.
8. Boehme KA, Nitsch J, Riester R, Handgretinger R, Schleicher SB, Kluba T, et al. Arsenic trioxide potentiates the effectiveness of etoposide in Ewing sarcomas. International journal of oncology. 2016;49(5):2135-46.
9. Mao J, Ma L, Shen Y, Zhu K, Zhang R, Xi W, et al. Arsenic circumvents the gefitinib resistance by binding to P62 and mediating autophagic degradation of EGFR in non-small cell lung cancer. Cell death & disease. 2018;9(10):963.
10. Modak S, Zanzonico P, Carrasquillo JA, Kushner BH, Kramer K, Cheung NK, et al. Arsenic Trioxide as a Radiation Sensitizer for 131I-Metaiodobenzylguanidine Therapy: Results of a Phase II Study. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2016;57(2):231-7.
11. Li GX, Pei QL, Gao Y, Liu KM, Nie JS, Han G, et al. Protective effects of hepatocellular canalicular conjugate export pump (Mrp2) on sodium arsenite-induced hepatic dysfunction in rats. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie. 2007;58(6):447-53.
12. Gregus Z, Gyurasics A, Csanaky I. Biliary and urinary excretion of inorganic arsenic: monomethylarsonous acid as a major biliary metabolite in rats. Toxicological sciences : an official journal of the Society of Toxicology 2000; 56(1): 18-25.
13. Zhang S, Ma C, Pang H, Zeng F, Cheng L, Fang B, et al. Arsenic trioxide suppresses cell growth and migration via inhibition of miR-27a in breast cancer cells. Biochemical and biophysical research communications. 2016;469(1):55-61.

14. Chang YW, Chen MW, Chiu CF, Hong CC, Cheng CC, Hsiao M, et al. Arsenic trioxide inhibits CXCR4-mediated metastasis by interfering miR-520h/PP2A/NF-kappaB signaling in cervical cancer. Annals of surgical oncology. 2014;21 Suppl 4:S687-95.
15. Tang L, Wang Y, Wang H, Xu B, Ji H, Xu G, et al. Long noncoding-RNA component of mitochondrial RNA processing endoribonuclease is involved in the progression of cholangiocarcinoma by regulating microRNA-217. Cancer science. 2019;110(7):2166-79.
16. Xu F, Yan JJ, Gan Y, Chang Y, Wang HL, He XX, et al. miR-885-5p Negatively Regulates Warburg Effect by Silencing Hexokinase 2 in Liver Cancer. Molecular therapy Nucleic acids. 2019;18:308-19.
17. Li, C, Wang, X, and Song, Q. MicroRNA 885-5p Inhibits Hepatocellular Carcinoma Metastasis by Repressing AEG1. OncoTargets and therapy ,2020, 13, 981-988
18. Sen S, Kundu G, Mekhail N, Castel J, Misono K, Healy B. Myotrophin: purification of a novel peptide from spontaneously hypertensive rat heart that influences myocardial growth. The Journal of biological chemistry 1990; 265(27): 16635-43.
19. Kim EY, Lee SS, Shin JH, Kim SH, Shin DH, Baek SY. Anticancer effect of arsenic trioxide on cholangiocarcinoma: in vitro experiments and in vivo xenograft mouse model. Clinical and experimental medicine 2014; 14(2): 215-24.
20. Gao J, Wang G, Wu J, Zuo Y, Zhang J, Jin X. Skp2 Expression Is Inhibited by Arsenic Trioxide through the Upregulation of miRNA-330-5p in Pancreatic Cancer Cells. Molecular therapy oncolytics 2019; 12: 214-23.
21. Bao X, Ren T, Huang Y, Wang S, Zhang F, Liu K, et al. Induction of the mesenchymal to epithelial transition by demethylation-activated microRNA-125b is involved in the anti-migration/invasion effects of arsenic trioxide on human chondrosarcoma. Journal of experimental & clinical cancer research : CR. 2016;35(1):129.
22. Zhu L, Huang F, Deng G, Nie W, Huang W, Xu H, et al. MicroRNA-212 targets FOXA1 and suppresses the proliferation and invasion of intrahepatic cholangiocarcinoma cells. Experimental and therapeutic medicine. 2017;13(5):2109.
23. Li H, Zhou ZQ, Yang ZR, Tong DN, Guan J, Shi BJ, et al. MicroRNA-191 acts as a tumor promoter by modulating the TET1-p53 pathway in intrahepatic cholangiocarcinoma. Hepatology (Baltimore, Md). 2017;66(1):136-51.
24. Borgmastars E, de Weerd HA, Lubovac-Pilav Z, Sund M. miRFA: an automated pipeline for microRNA functional analysis with correlation support from TCGA and TCPA expression data in pancreatic cancer. BMC bioinformatics 2019; 20(1): 393.
25. Liu Y, Bao Z, Tian W, Huang G. miR-885-5p suppresses osteosarcoma proliferation, migration and invasion through regulation of beta-catenin. Oncology letters 2019; 17(2): 1996-2004.
26. Afanasyeva EA, Mestdagh P, Kumps C, Vandesompele J, Ehemann V, Theissen J, et al. MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. Cell death and differentiation. 2011;18(6):974-84.

27. Hussein NA, Kholy ZA, Anwar MM, Ahmad MA, Ahmad SM. Plasma miR-22-3p, miR-642b-3p and miR-885-5p as diagnostic biomarkers for pancreatic cancer. Journal of cancer research and clinical oncology 2017; 143(1): 83-93.
28. Gui J, Tian Y, Wen X, Zhang W, Zhang P, Gao J, et al. Serum microRNA characterization identifies miR-885-5p as a potential marker for detecting liver pathologies. Clinical science (London, England : 1979). 2011;120(5):183-93.
29. Khan SQ, Kelly D, Quinn P, Davies JE, Ng LL. Myotrophin is a more powerful predictor of major adverse cardiac events following acute coronary syndrome than N-terminal pro-B-type natriuretic peptide. Clinical science (London, England : 1979) 2007; 112(4): 251-6.
30. Anderson KM, Berrebi-Bertrand I, Kirkpatrick RB, McQueney MS, Underwood DC, Rouanet S, et al. cDNA sequence and characterization of the gene that encodes human myotrophin/V-1 protein, a mediator of cardiac hypertrophy. Journal of molecular and cellular cardiology. 1999;31(4):705-19.
31. O'Brien RJ, Loke I, Davies JE, Squire IB, Ng LL. Myotrophin in human heart failure. Journal of the American College of Cardiology 2003; 42(4): 719-25.
32. Jafarian A, Taghikani M, Abroun S, Allahverdi A, Lamei M, Lakpour N, et al. The Generation of Insulin Producing Cells from Human Mesenchymal Stem Cells by MiR-375 and Anti-MiR-9. PloS one. 2015;10(6):e0128650.
33. Li Y, Xu X, Liang Y, Liu S, Xiao H, Li F, et al. miR-375 enhances palmitate-induced lipoapoptosis in insulin-secreting NIT-1 cells by repressing myotrophin (V1) protein expression. International journal of clinical and experimental pathology. 2010;3(3):254-64.
34. Muniz Lino MA, Palacios-Rodriguez Y, Rodriguez-Cuevas S, Bautista-Pina V, Marchat LA, Ruiz-Garcia E, et al. Comparative proteomic profiling of triple-negative breast cancer reveals that up-regulation of RhoGDI-2 is associated to the inhibition of caspase 3 and caspase 9. Journal of proteomics. 2014;111:198-211.
35. Das B, Gupta S, Vasanji A, Xu Z, Misra S, Sen S. Nuclear co-translocation of myotrophin and p65 stimulates myocyte growth. Regulation by myotrophin hairpin loops. The Journal of biological chemistry 2008; 283(41): 27947-56.

Supplementary Figure Legends

Fig.S1. The filter of miRNAs:(A) Heatmap of differentially expressed microRNAs (B): Six microRNAs were selected as the detection target of cells and tissues according to the difference multiple.

Fig.S2. The filtered mRNAs

Fig.S3. IC50 of the cells transfected with miR-196a-5p inhibitor or not:(A) RBE and HCCC-9810 were transfected by scrambled or miR-196a-5p inhibitor, IC50 of 5-FU(top) or CDDP(bottom) in them were analyzed.

Fig.S4. The effect of MTPN siRNAs

Fig.S5. The primers

Figures

Fig.1

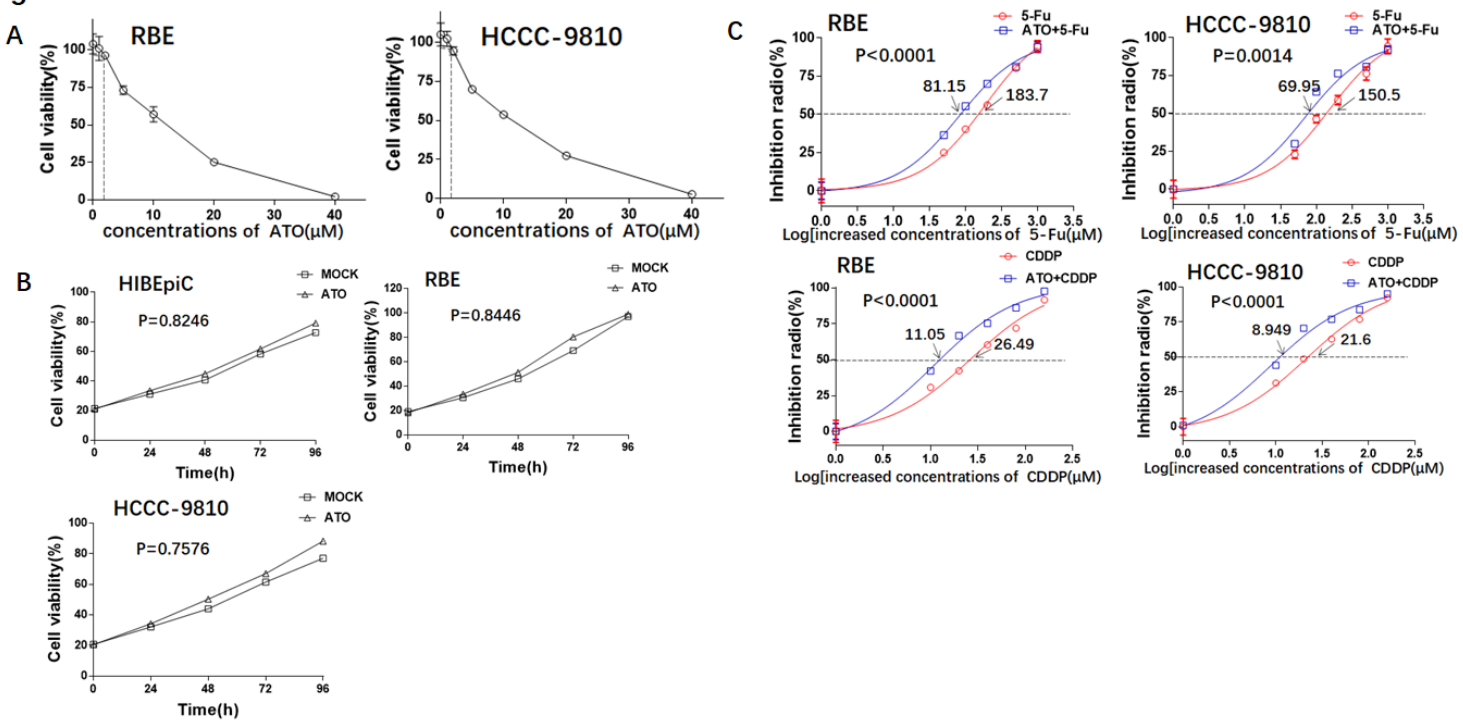

Figure 1

Sensitization effect of ATO in cholangiocarcinoma cells: (A) Cell viability of RBE and HCCC-9810 were analyzed by CCK-8 solution after ATO treatment. (B) Time gradient effect of 2 μM ATO on the activity of HIBEpic, RBE and HCCC-9810 were analyzed by CCK-8 solution. (C) With 2 μM ATO or not ,IC50 of 5-Fu and CDDP in RBE and HCCC-9810 were analyzed.

Fig.2

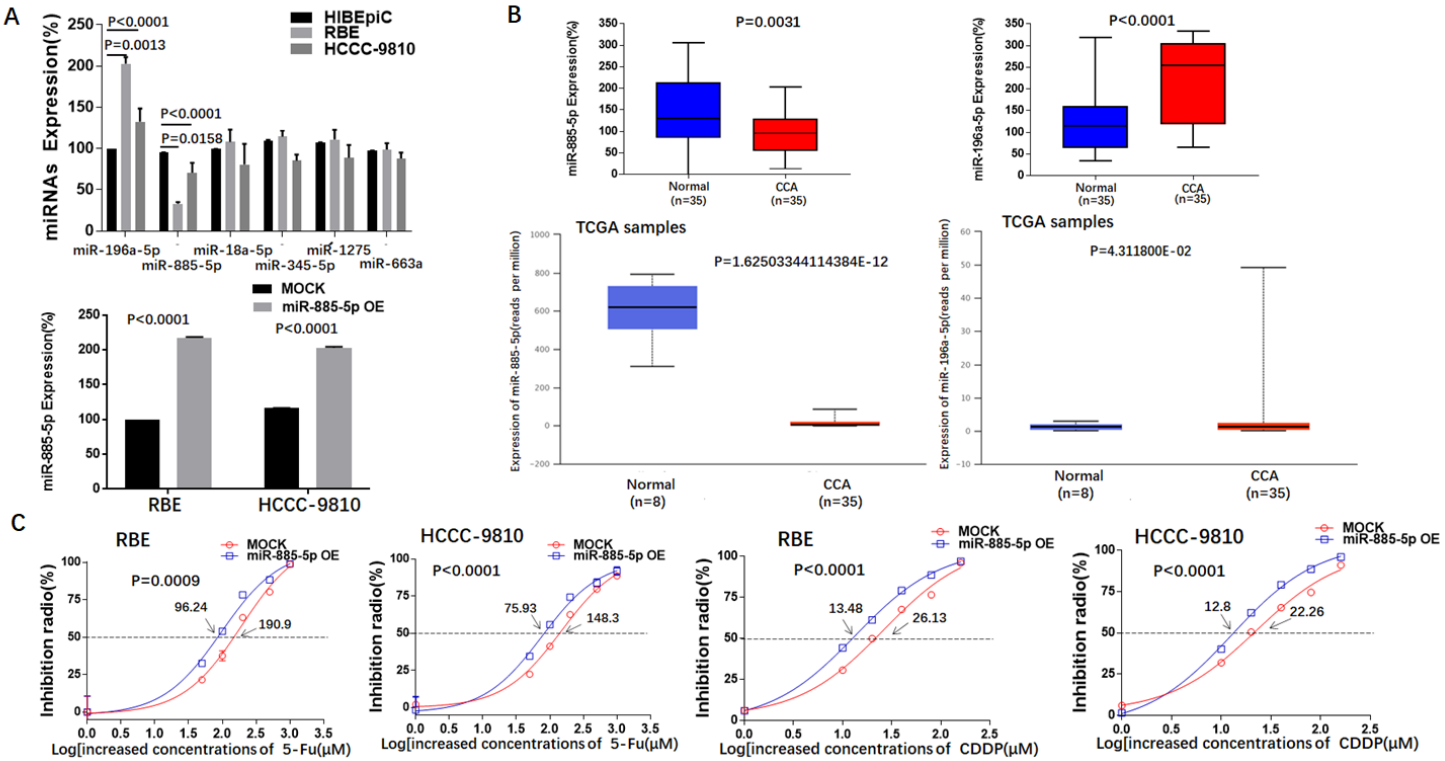

Figure 2

Effect of miR-885-5p on chemosensitivity of cholangiocarcinoma cell lines: (A) The expression levels of six differentially expressed microRNAs in HIBEpic, RBE and HCCC-9810 were analyzed by qPCR. (B) Expression of miR-885-5p/miR-196a-5p in 35 pairs of CCA and normal tissues (top)/TCGA samples (bottom) were analyzed by qPCR. (C) RBE and HCCC-9810 were transfected by scrambled or miR-885-5p mimic, IC50 of 5-FU (left) or CDDP (right) in them were analyzed.

Fig.3

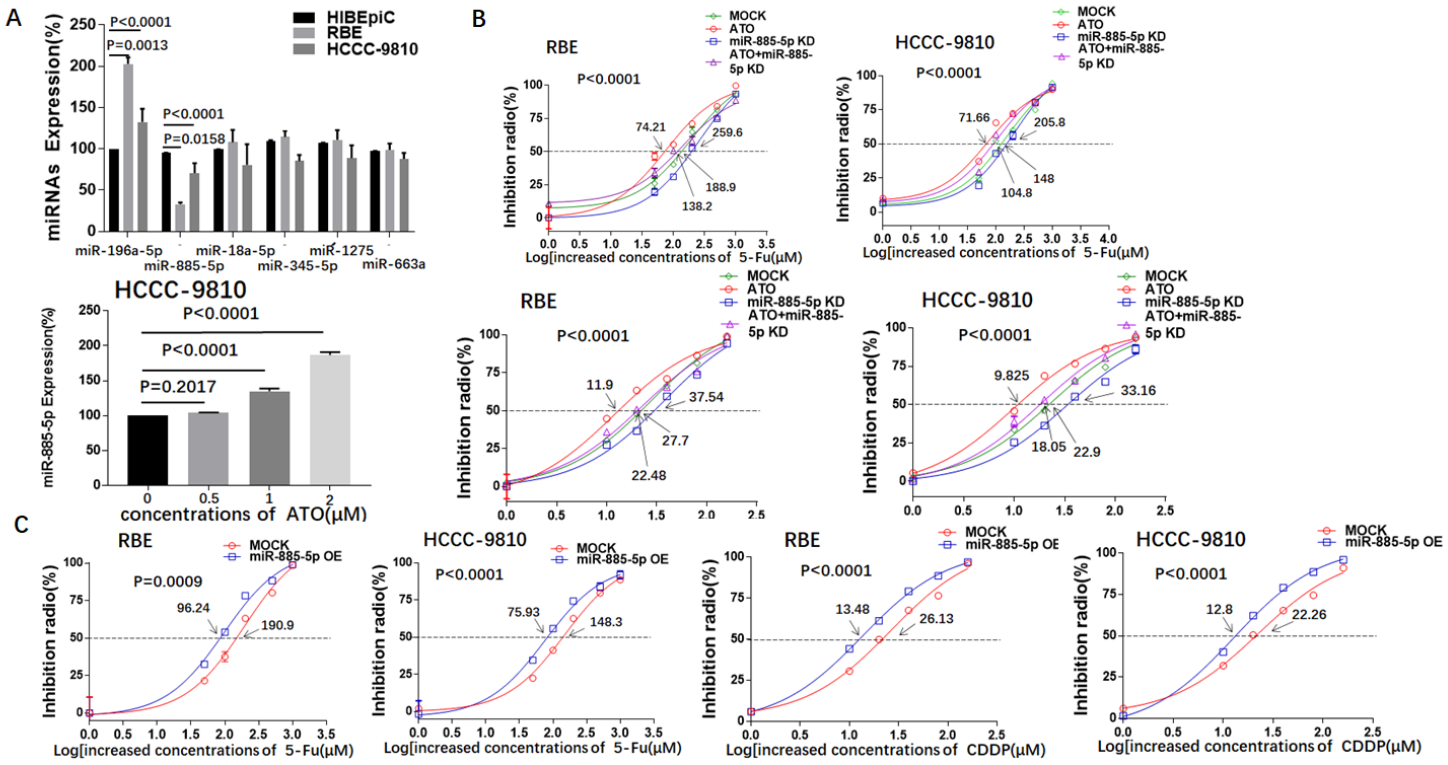

Figure 3

ATO enhanced the chemosensitivity of cholangiocarcinoma cells through miR-885-5p: (A) Expression of miR-885-5p in RBE and HCCC-9810 treated with ATO at 0, 0.5, 1, 2μM were analyzed by qPCR. (B) Rescue experiment, RBE and HCCC-9810 were transfected by scrambled or miR-885-5p inhibitor, then treated with ATO or not, the IC50 of 5-Fu and CDDP in the cells were analyzed.

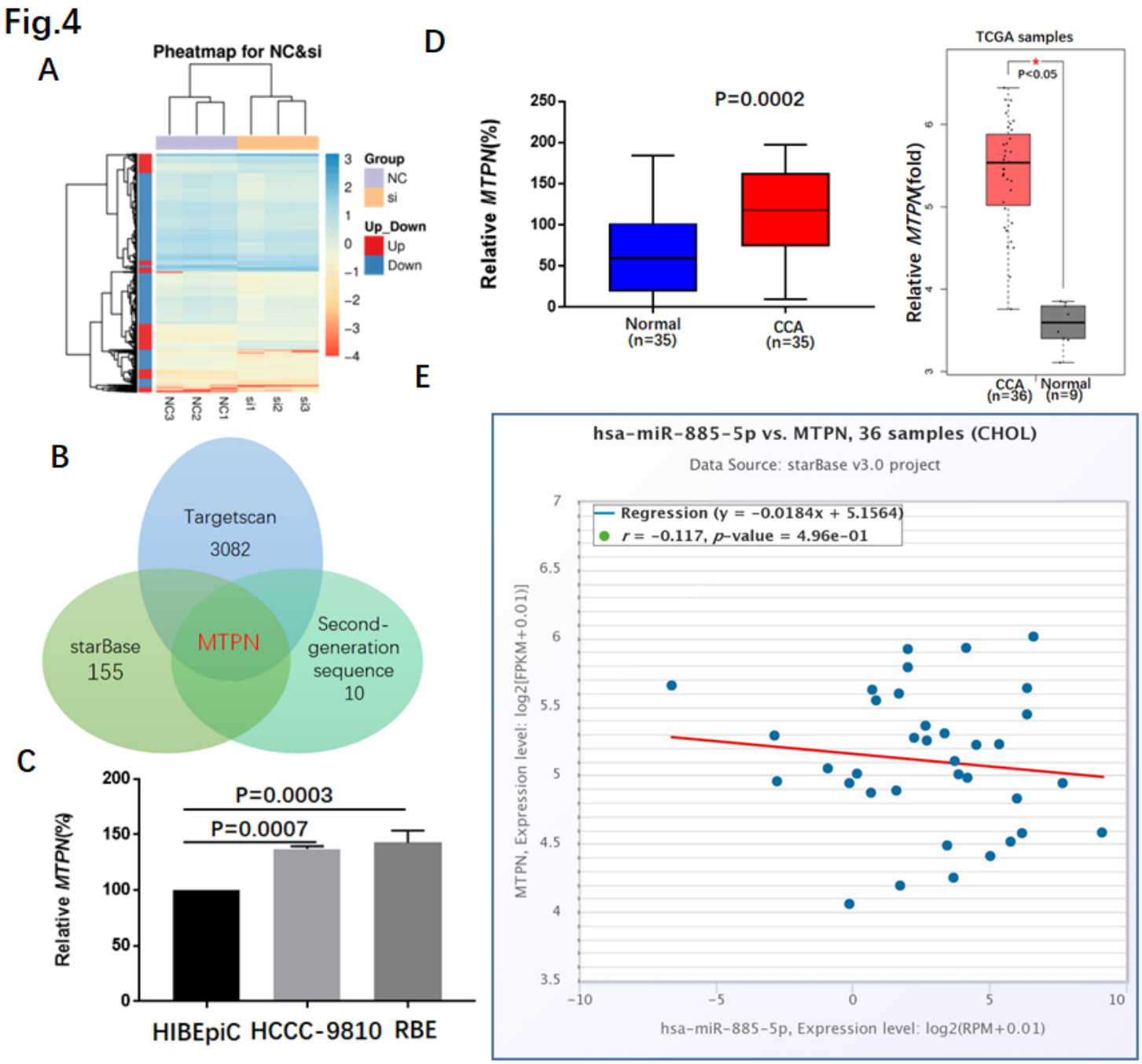

Figure 4

MTPN was a target gene of miR-885-5p in CCA: (A) Heatmap of all detected mRNAs. (B) MTPN was the intersection of prediction results of Targrtscan and starBase combined with second-generation sequence. (C) Expression of MTPN in HIBEpic, RBE and HCCC-9810 were analyzed by qPCR .(D) Expression of MTPN in 35 pairs of CCA and normal tissues (left)/TCGA samples (right) were analyzed by qPCR. (E) The analysis of the correlation between miR-885-5p and MTPN in 36 samples in starBase.

Fig.5

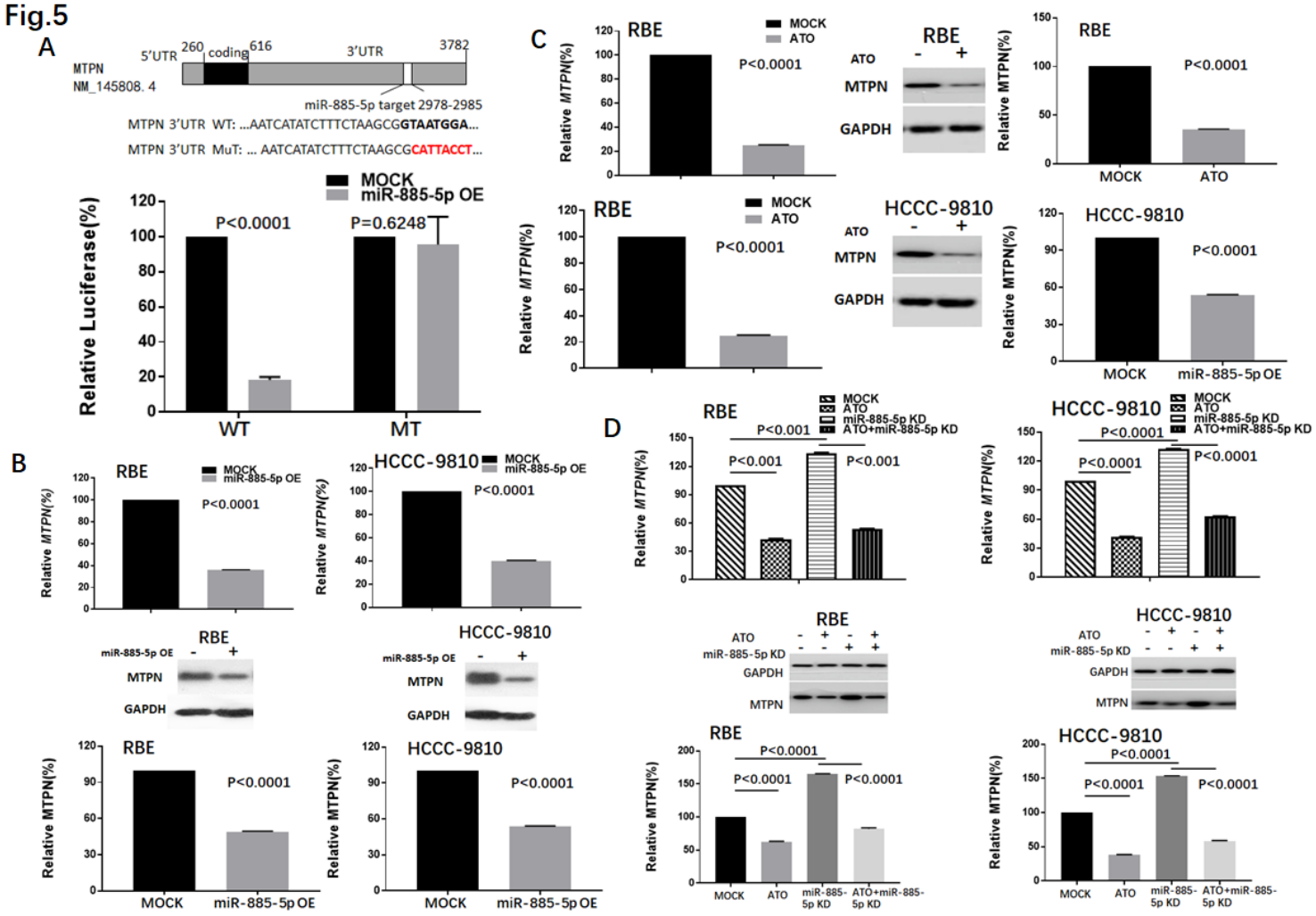

Figure 5

ATO regulated the expression of MTPN through miR-885-5p: (A) Luciferase reporter gene experiment showed that miR-885-5p directly combined with wild-type MTPN to regulate its expression. (B) qPCR and IB analysis of expression of MTPN mRNA (top) and protein (bottom) in RBE and HCCC-9810 ,which transfected with scramed or miR-885-5p mimic. (C) qPCR and IB analysis of expression of MTPN mRNA (top) and protein (bottom) in RBE and HCCC-9810 ,which treated with ATO or not. (D) Rescue experiment, RBE and HCCC-9810 were transfected by scrambled or miR-885-5p inhibitor , then treated with ATO or not. qPCR and IB analysis of expression of MTPN mRNA (top) and protein (bottom) in RBE and HCCC-9810.

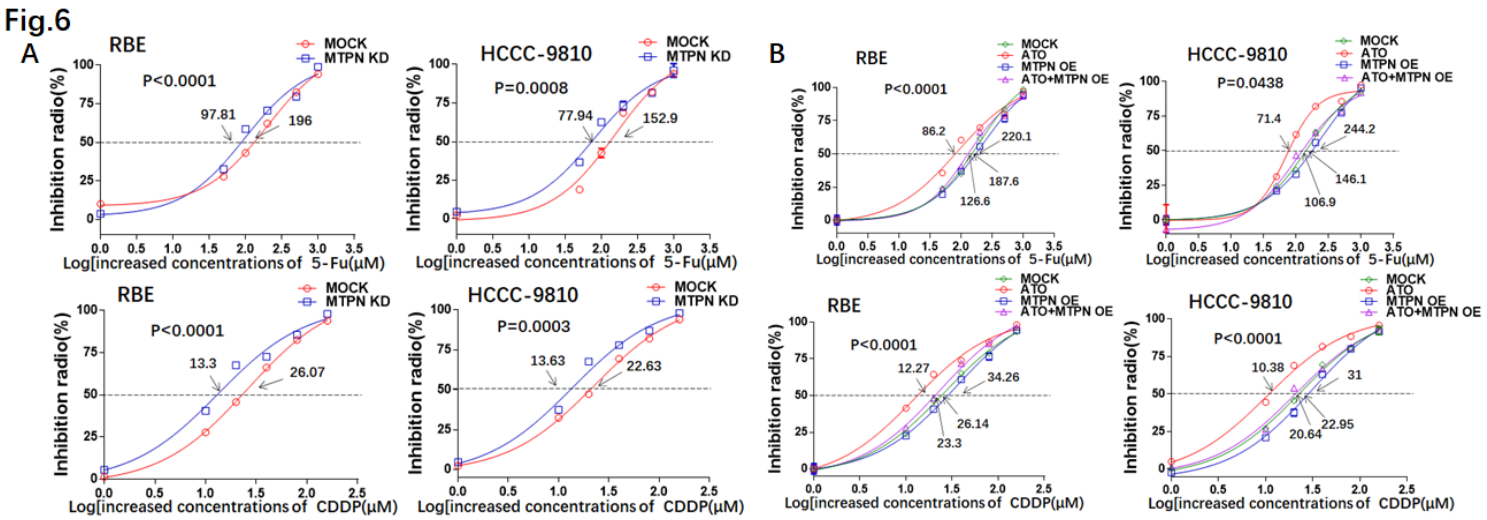

Figure 6

ATO/miR-885-5p/MTPN axis improved the anti-drug-resistance of cholangiocarcinoma cells: (A) IC50 of 5-Fu and CDDP in RBE and HCCC-9810, which transfected with scrambled or MTPN siRNA. (B) Rescue experiment, RBE and HCCC-9810 were transfected by scrambled or MTPN plasmid, then treated with ATO or not. the IC50 of 5-Fu and CDDP in the cells were analyzed.

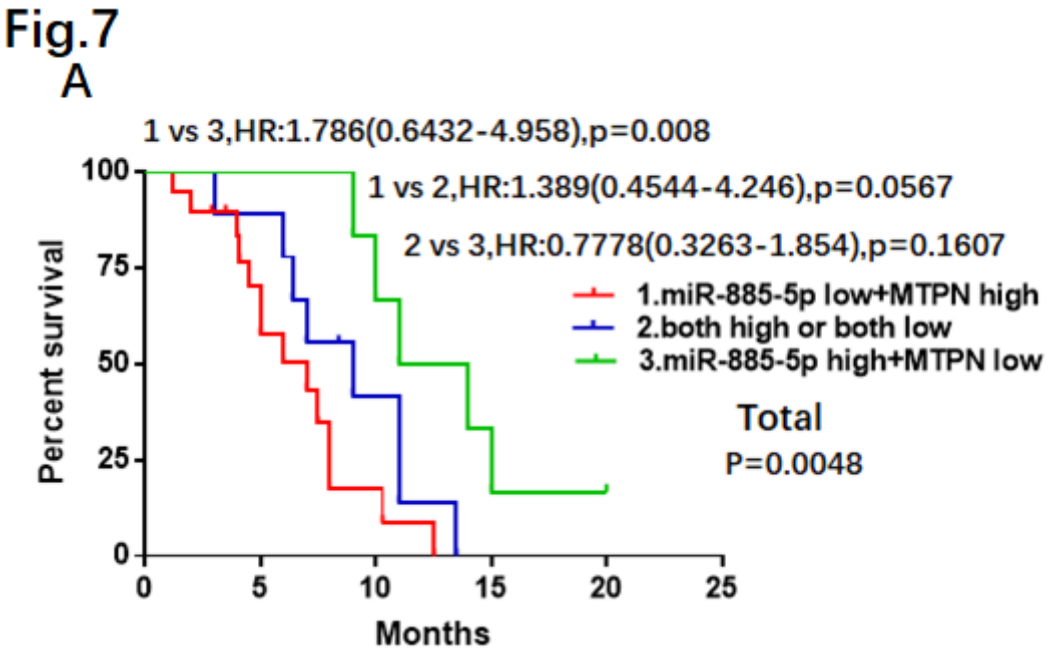

Figure 7

Validation of prognostic values of miR-885-5p and MTPN in CCA: Kaplan-Meier analysis of the prognostic significances of miR-885-5p and MTPN.

Supplementary Files

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