MiR-155-5p Suppress SOX1 to Promote the Proliferation of Cholangiocarcinoma via RAF/MEK/ERK Pathway

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Abstract

Background

Accumulating evidences indicate that SOX1 is closely related to tumorigenesis and development, upregulation of SOX1 is recently reported to suppress growth of human cancers. However, the expression and role of SOX1 in cholangiocarcinoma (CCA) remain unknown.

Methods

The expression levels of SOX1 in CCA tissues and normal bile duct tissues were examined by public GEO database, and western blot and immunohistochemistry were used to confirm the expression again. Cell proliferation assay (CCK-8) and colony formation assay were performed to determine proliferation of CCA cells. A model of transplatable subcutaneously tumors in mouse was used to evaluated proliferation of CCA in vivo. The putative regulating factor of SOX1 were disclosed by Targetscan and a dual-luciferase reporter assay.

Results

SOX1 was downregulated in CCA tissues. Overexpression of SOX1 significantly inhibited cell proliferation in vitro and tumor growth in vivo. Furthermore, miR-155-5p directly targets 3'UTR of SOX1 and inhibits expression of SOX1, resulting in the activation of RAF, MEK and ERK phosphorylation and thus CCA proliferation. However, when SOX1 expression was restored in miR-155-5p overexpressing cell lines, the phosphorylation level of RAF, MEK and ERK were decreased, as well as the proliferation of CCA cells.

Conclusion

MiR-155-5p could bind to the 3'UTR of SOX1 to decrease the expression of SOX1, and further activated the RAF/MEK/ERK signaling pathway to promote CCA progression.

Background

Cholangiocarcinoma (CCA) is reported to be a malignant tumor originating from the epithelium of the bile duct. The prognosis of CCA is extremely poor, the 5-year survival rate is less than 10%, surgery is the best curative treatment for it. However, CCA patients are often diagnosed at an advanced stage of cancer, fewer than 30% have an opportunity of surgery(1-3). For the rest of the patients, only chemotherapy is available, but the effect is limited. Epidemiological evidence shows that the incidence of CCA is increasing over the past decades(4, 5). Based on the reasons mentioned above, exploring the development mechanisms of CCA and finding novel biomarkers and therapeutic targets are urgently needed.

SOX (SRY-related HMG-box) domain proteins are a conserved group of transcriptional regulators defined by the presence of a highly conserved high mobility group (HMG) domain that mediates DNA binding, and they are related to the mammalian testis determining factor gene sex-determining region Y (SRY)(6). *SOX1* is one of the 20 *SOX* genes that have been identified in the mammalian Genome, and it plays an important role in embryonic development and regulation of stem cells(7, 8). The SOX family is closely related to tumorigenesis and development, for example: SOX2 interacts with BCL11A to control the development of lung squamous carcinoma(9); hypoxia may increase the hepatocellular carcinoma cancer (HCC) stem cells population via altering the AR/miR-520f-3p/SOX9 signaling(10); SOX17 is involved in the p53-mediated apoptosis pathway, and increases the sensitivity of endometrial cancer cells to cisplatin(11). It has been reported that SOX1 can inhibit the occurrence and development of HCC, esophageal cancer, nasopharyngeal carcinoma, lung cancer and cervical cancer(7, 12-14), but its role in CCA has not been reported. Based on these, our study focused on SOX1 to explore its effect on CCA.

In this study, we found that SOX1 was downregulated in CCA, and overexpression of SOX1 could inhibit the proliferation of CCA in vitro and vivo. MiR-155-5p could inhibit SOX1 expression by combining with 3'UTR of SOX1, and activate the RAF/MEK/ERK pathway to promote CCA progression. Our findings demonstrate the critical role of miR-155-5p/SOX1/RAF/MEK/ERK axis in CCA progression, which may provide a novel diagnostic and therapeutic target for CCA.

Methods

Cell lines, miRNA transfection and lentivirus infection

Human CCA cell line TFK-1 was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). HUCCT-1 cell line was purchased from the RIKEN bioresource Center (Saitama-ken, Japan). The TFK-1 and HUCCT-1 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in a humidified incubator containing 5% CO2 at 37 °C. HEK293T cells were cultured in DMEM supplemented with 10% FBS. Lentivirus for overexpressing SOX1 and control lentivirus (Vector) were purchased from Genechem (Shanghai, China). Cells were infected with lentivirus particles for 24h, then cultured in fresh medium. Stable cell lines were generated via puromycin (5 μg/ml) selection. All miRNA oligos were purchased from Guangzhou RiboBio: miR-200b-3p: UAAUACUGCCUGGUAAUGAUGA; miR-155-5p: UUAAUGCUAAUCGUGAUAGGGGUU; miR-144-3p: UACAGUAUAGAUGAUGUACU; miR-NC: UUUGUACUACACAAAAGUACUG. miRNA transfection was performed using Lipofectamine 2000 (Invitrogen) as per manual.

Immunohistochemistry

A total of 9 CCA specimens and 4 normal bile duct tissues were collected from Tongji Hospital of Huazhong University of Science and Technology (HUST). The tissue samples were obtained with the approval by the Ethical Committee of HUST and patient's written informed consent. The samples were formalin

fixed, paraffin embedded on slides, and characterized according to standard pathology. Immunohistochemistry was performed on 3-μm serial sections as described previously (REF). In brief, the sections were subjected to deparaffinization and hydration steps followed by quenching and peroxidase reaction steps. Antigen retrieval was performed by microwave irradiation for 8 min in citric acid (0.01M). Following by one hour of blocking in 10% goat serum, tissue sections were immunostained with SOX1 antibody (1:200), incubated with secondary antibody, followed by 3,3-Diaminobenzidine tetrahydrochloride (DAB) staining, and then counterstained with hematoxylin.

Bioinformatic analysis

Two independent CCA gene expression profiles GSE32225 (which includes 149 cholangiocarcinoma samples and 6 normal biliary epithelial cell samples) (15) and GSE76297 (which includes 91 cholangiocarcinoma samples and 92 non-tumor tissue samples)(16) were taken from the Gene Expression Omnibus database (GEO). GSE32225 is based on the platform Illumina HumanRef-8 WG-DASL v3.0 (GPL8432), and GSE76297 is based on the platform Affymetrix Human Transcriptome Array 2.0 (GPL17586). SOX1 gene expression values (log2) were normalized across all CCA lines. One-way analysis of variance (ANOVA) between groups of patients was performed using Graphpad Prism (version 7.0, San Diego, CA).

Western blot

Cells were lysed in RIPA lysis buffer (50mM Tris-HCl, 150mM NaCl, 1 mM EDTA-Na2, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitors and phosphatase inhibitor on ice for 15 min. The cell lysates were cleared by centrifugation at 12,000 × g for 10 min, then heat-denatured with 5 x Laemmli buffer (G2013, Servicebio). The prepared samples with equal amount of total protein (20 μg) were subjected to SDS-PAGE and immunoblotting following the standard procedure. The following antibodies were used: anti-SOX1 (ab109290, Abcam), anti-β-catenin (ab32572, Abcam), anti-HES1 (#11988, Cell Signaling Technology [CST]), anti-PROX1 (11067-2-AP, Proteintech), anti-p38 (#8690, CST), anti-phospho-p38 (#9215, CST), anti-ERK (#4695, CST), anti-phospho-ERK (#4370, CST), anti-AKT (#4691, CST), anti-phospho-AKT (#4060, CST), anti-JNK (#9252, CST), anti-phospho-JNK (#700031, Invitrogen), anti-RAF (#9422, CST), anti-phospho-RAF (#9427, CST), anti-MEK (51080-1-AP, Proteintech), anti-phospho-MEK (#9154, CST), anti-α-Tubulin (11224-1-AP, Proteintech), anti-GAPDH (BM1623, boster).

Cell proliferation assay and colony formation assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) (Dojindo, Tokyo, Japan). For colony formation assay, cells were cultured in 3cm-dishes for 2 weeks, fixed with 4% paraformaldehyde for 30 min, then stained with 1% crystal violet for 30 min. Colonies with diameter greater than 100 μm were counted.

Xenografts

Male BALB/C nude mice at 4 weeks of age were obtained from the Beijing Vital River Laboratory Animal Technology, Beijing, China and housed in a pathogen-free facility. For mouse xenograft models, TFK-1 cells (2 × 105 per mouse) expressing either SOX1 or negative control vector in 200ul PBS were implanted subcutaneously into the right flank of the mice. Mice were sacrificed 18 days post inoculation, tumor volumes (in cubic millimeter) were measured a digital Vernier caliper and calculated according to the width2 × length/2 formula. This study was performed according to protocols approved by the Experimental Animal Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology.

Luciferase reporter activity assay

The psiCHECK-2 luciferase reporter vector was purchased from Promega. PCR-mediated site-directed mutagenesis was used to generate mutation as indicated. The SOX1-WT-3'UTR and SOX1-Mutant-3'UTR luciferase reporter constructs were generated by inserting either wildtype or mutant 400bp fragments of the predicted 3'UTR region of human SOX1 gene into the XhoI and NOTI multi-clonal sites of psiCHECK-2 vector.

The sequences of inserted 3'UTR region were listed below:

SOX1-WT, 5'-
GCTAACCGATGTGAACGCAAAATGCCTTGTTCATTATTCCTGACGAGATCTTGAGGTTGTTTGATGCTTTAAATTTTTTAATTATATTATTTTCTAGGTGTTTATTGGTACA
3'; SOX1-mut, 5'-
GCTAACCGATGTGAACGCAAAATGCCTTGTTCATTATTCCTGACGAGATCTTGAGGTTGTTTGATGCTTTAAATTTTTTAATTATATTATTTTCTAGGTGTTTATTGGTACA
3'.

HEK293T cells were co-transfected with indicated plasmids and miR-155-5p or miR-NC for 48h. Firefly and Renilla luciferase activities were measured using the dual luciferase reporter assay system (Promega) according to the manufacturer's protocol.

Statistical analysis

All data are expressed as the mean ± S.D. The difference between two independent samples was tested by two-tail Student's t-test and the difference among the groups was determined by two-tail ANOVA using GraphPad Prism 5.0 (GraphPad Prism Software, San Diego, CA, USA). A p value of < 0.05 was considered significant.

Results

SOX1 is down-regulated in CCA tissues

To investigate the functions of SOX1 in CCA, we firstly examined the expression of SOX1 in CCA patients from public GEO database (GSE32225, GSE76297). We found that SOX1 was downregulated in CCA, compared with the normal bile duct tissues (Figure1A and 1B). Then we further detected the protein level of SOX1 in 13 clinical samples included 9 CCA and 4 normal bile duct tissues using immunohistochemistry and western blot and verified the results from public database. The results showed that SOX1 protein expression was significantly downregulated in primary CCAs compared with normal bile duct tissues (Figure1C and 1D), which were consist with the above results.

Overexpression of SOX1 inhibits CCA cells proliferation in vitro and tumor growth in vivo

Because the rapid growth of tumor is the main cause of poor prognosis, we thought to investigate the impact of SOX1 on growth of CCA. We checked the proliferation-suppressive effect through overexpression of SOX1 in TFK-1 and HUCCT-1 through lentiviral mediated transfection carrying SOX1 and negative control vectors. Western blot was performed to confirm that SOX1 was over-expressed stably in TFK-1 and HUCCT-1(Figure 2A). Then we assessed the effect of SOX1 on cells proliferation using plate clone assay and Cell Counting Kit-8 (CCK-8). As shown in Figure 2B-2D, CCA cells with overexpression of SOX1 had lower proliferation rate and fewer colony numbers than negative control cells. Furthermore, the subcutaneous tumor growth of TFK-1 stably overexpressing SOX1 and TFK-1 transfected with negative control vector in BALB/C nude mice is shown in Figure 2E. Clearly, the tumor volume in mice injected with SOX1-overexpressed TFK-1 were significantly smaller than the negative control group ($P < 0.05$). Overall, these results suggested that SOX1 inhibits the proliferation of CCA cells in vitro and tumor growth in vivo.

MicroRNA-155-5p directly targets 3'UTR of SOX1 and inhibits expression of SOX1

To elucidate the mechanisms underlying the regulation of SOX1, we focused on microRNA, which has been proved to play an important role in a variety of tumors. One of the classic mechanisms of miRNA is that it can specifically bind to the 3'UTR region of the target gene, such binding inhibits the translation and reduces the stability of the target mRNA, leading to decreased expression of the target protein(17). We used Targetscan (http://www.targetscan.org/vert_71/) to screen out a variety of candidate miRNAs and chose the top three miRNA (miR-200b-3p, miR-144-3p, miR-155-5p) as candidates (Figure 3A and 3B). Then we transfected the mimic and inhibitor of these candidate miRNAs to TFK-1 and HUCCT-1 and detected the expression of SOX1. The results of western blot showed that neither the miR-200b-3p nor the miR-144-3p could reduce the protein level of SOX1. On the other hand, only miR-155-5p significantly reduced the expression of SOX1 (Figure 3C). Then we found that miR-155-5p might bind to the 3'-untranslated regions (3'UTR site of SOX1. (Figure 3D). We introduced the wild-type (WT) or mutant-type (MT) 3'UTR of SOX1 into psiCHECK2 plasmids (Figure 3E). The luciferase activity assay suggested that the mimic of miR-155-5p successfully decreased the luciferase activity when it added to the WT, however, the luciferase activity of mimic which was added to MT showed no obvious change (Figure 3F). Overall, these results showed that miR-155-5p could suppress the expression of SOX1 through binding to its 3'UTR region.

MiR-155-5p inhibits SOX1 to activate the RAF/MEK/ERK pathway

According to the above experimental results, SOX1 can inhibit the proliferation of CCA. In order to further explore its internal mechanisms, we screened the downstream signaling pathway of SOX1. Guan, Z et al. proposed that SOX1 could interact with β - Catenin in Wnt signaling pathway and reversed the malignant biological behaviors of Nasopharyngeal carcinoma by inhibiting the activation of β-Catenin(7); SOX1 could interact with hes1, which is the downstream target gene of notch1. Interestingly, Zhou, X. Y., et al. proposed that TRIP13 could promote the proliferation and invasion of epithelial ovarian cancer through Notch signaling pathway(18); Liao, B. et al. proposed that hepatitis B virus X protein could activate the Notch pathway, thus promoting the development of liver cancer(19); Li, W. J., et al. proposed that miR-1179 could inhibit the metastasis of breast cancer by inhibiting Notch signaling pathway and Hes1(20). In addition, SOX1 can block cell cycle exit by Prox1, which leads to neuron differentiation in vitro and in vivo(21), Hogstrom, j et al. Suggested that Prox1 might play a role by inhibiting Notch signaling pathway in colon cancer(22). To further explore the mechanisms of SOX1 in CCA, we overexpressed SOX1 in CCA cells, and then detected the expression of two key proteins interacting with SOX1 (hes1, prox1) and the signaling pathways related to cholangiocarcinoma (AKT, JNK, P38, ERK) by Western blot. Interestingly only the phosphorylation level of ERK changed significantly, whereas the expression level of β-catenin, prox1, hes1 and phosphorylation level of AKT, JNK, P38 remained stable. These observations suggested that overexpression of SOX1 decrease the phosphorylation level of ERK, while the total ERK expression had no significant change (Figure 4A).

Through the above experiments, we have proved that miR-155-5p suppressed the expression of SOX1, SOX1 suppress CCA via ERK pathway, we hypothesized that miR-155-5p inhibited SOX1 to promote CCA also via ERK signaling pathway. To address it, we transfected miR-155-5p and miR-155-5p inhibitor to CCA cells. The result showed that miR-155-5p could activate the ERK pathway by increasing the phosphorylation of ERK in HUCCT-1 (Figure 4B).

As we know, ERK signaling pathway is one of the mitogen-activated protein kinase (MAPK) signaling pathways. Phosphorylation can lead to activation of ERK, which can promote cell proliferation, invasion and metastasis in cancer. Downstream of KRAS, the RAF→MEK→ERK signaling pathway plays a central role in carcinogenesis(23). Western blot showed miR-155-5p could also enhance the phosphorylation of RAF and MEK. Importantly, reintroduction of SOX1 reversed the phenomenon of high phosphorylation level of RAF, MEK and ERK induced by mir-155-5p (Figure 4C). These data indicated that miR-155-5p might suppress SOX1 to active RAF/MEK/ERK signaling pathway.

MiR-155-5p promotes the proliferation of CCA cells via inhibiting the expression of SOX1

To study the role of miR-155-5p in CCA cells, we performed CCK-8 and plate clone assay. As we expected, miR-155-5p promoted the proliferation of CCA cells, however, SOX1 overexpression blocked the proliferation effects of miR-155-5p on CCA cells, indicating that downregulation of SOX1 is an important mechanism for miR-155-5p to promote cell proliferation (Figure 5A, 5B and 5C). These data indicated that miR-155-5p might promote the proliferation of CCA cells via inhibiting the expression of SOX1.

Discussion

SOX1 is a member of the transcription factor sox family, which plays an important role in embryo and postnatal development. According to the current research, SOX1 also has a certain inhibitory effect on a variety of tumors, and many studies have proved that SOX1 can be used as a biomarker for a variety of tumors, such as cervical cancer, colon cancer, glioma, liver cancer, ovarian cancer, small cell lung cancer(24-29), etc. But association between SOX1 and CCA has not been reported. In this study, we firstly found that SOX1 was downregulated in CCA tissues. Then we confirmed that SOX1 significantly suppressed CCA proliferation and growth in vitro and in vivo. In addition, SOX1 might be regulated by miR-155-5p. Finally, we confirmed that miR-155-5p/SOX1/RAF/MEK/ERK axis might play an important role in CCA development (Figure 6).

To understand the role of SOX1 in CCA, we firstly examined the expression of SOX1 in CCA patients from public GEO database (GSE32225, GSE76297), which showed SOX1 was downregulated in CCA tissues. We used lentiviruses to change the expression of SOX1 in CCA cells, then CCK-8, plate clone assay and subcutaneous tumor-bearing mice were performed. These results showed that overexpression of SOX1 could significantly suppress CCA cells proliferation in vitro and vivo. These data indicated that SOX1 could inhibit the biological behavior of CCA.

Increasing studies have demonstrated that miRNAs played a crucial role in cancer cell proliferation. To explored the upstream factors regulating SOX1 expression in CCA, we began to focus on microRNA. Based the technology of bioinformatics analysis, we screen out a variety of candidate miRNAs and chose the top three miRNA (miR-200b-3p, miR-144-3p, miR-155-5p) as candidates. We performed western blot and luciferase activity assay to demonstrate our hypothesis. The observations showed that miR-155-5p might decrease the expression of SOX1 through binding to its 3'UTR region.

The potential molecular mechanism of SOX1 correlating with CCA was also studied in this research. According to previous studies, ERK could be activated to promote the development of colon cancer(30); CD110 could promote the progression of pancreatic cancer through activating the ERK(31); 1,25-$(OH)_2D_3$ could inhibit the proliferation, invasion and metastasis of breast cancer cells by inhibiting the activation of ERK signaling pathway(32). ERK expression is critical for tumor development and their hyperactivation plays a major role in cancer development and progression. The RAF/MEK/ERK pathway is the most important signaling cascade among all MAPK signal transduction pathways, and plays a crucial role in the survival and development of tumor cells. Ras can bind and translocate RAF from the cytoplasm to the cell membrane, where RAF is activated. Activated RAF-1 continues to activate downstream MEK and ERK, and then regulates the downstream gene expression(33). Interestingly, we founded that overexpression of SOX1 could decrease the phosphorylation of ERK in CCA cells. Then we explored the relationship between miR-155-5p and the pathway of RAF/MEK/ERK. We observed that overexpression of miR-155-5p could activate the RAF/MEK/ERK pathway by enhancing the phosphorylation of RAF/MEK/ERK, and the proliferation of CCA cells was increased. But when we increased the SOX1 expression in the cells which had had overexpressed miR-155-5p, the activation of the pathway was significantly decreased, and the proliferation of CCA cells was also restored. These data revealed that miR-155-5p could promote CCA through inhibiting SOX1 to active the RAF/MEK/ERK signaling pathway.

Conclusions

In summary, we found that SOX1 could suppress RAF/MEK/ERK pathway through decreasing the phosphorylation of RAF, MEK, ERK, and then inhibited the proliferation of CCA cells in vitro and tumor growth in vivo. MiR-155-5p could bind to the 3'UTR of SOX1 to decrease the expression of SOX1, and further activated the RAF/MEK/ERK signaling pathway to promote CCA progression. Hence, our findings demonstrate the critical role of miR-155-5p/SOX1/RAF/MEK/ERK axis in CCA progression, which may provide a novel therapeutic target for CCA.

Abbreviations

CCA, cholangiocarcinoma; SOX1, SRY-related HMG-box1; GEO, Gene Expression Omnibus; miRNA, microRNA; WT, wild type; MT, mutant type.

Declarations

Ethics approval and consent to participate: The work has been performed in accordance with Declaration of Helsinki. The research involving human participants were reviewed and approved by the Ethics Committee of Tongji Hospital. The animal study was reviewed and approved by the Committee on the Ethics of Animal Experiments of Tongji Hospital of Huazhong University of Science and Technology (Wuhan, China).

Consent for publication: Publication of this study was undertaken with all of the patients' informed.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. And all the data has been submitted as supplemental material to editors.

Competing interests: The authors declare that they have no conflict of interests.

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Authors' contributions: All authors, DW, FX, BW and YJC contributed to the conception and

design of the study. GHW acquired the samples. DW and YJC designed the experiments. DW and FX conducted the molecular studies and performed the experiments. GHW and WZL participated in the samples selection. DW, FX, GHW, WZL and BW contributed to the data analysis and interpretation. DW and YJC drafted the manuscript. All authors revised it critically and approved the final version.

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Figures

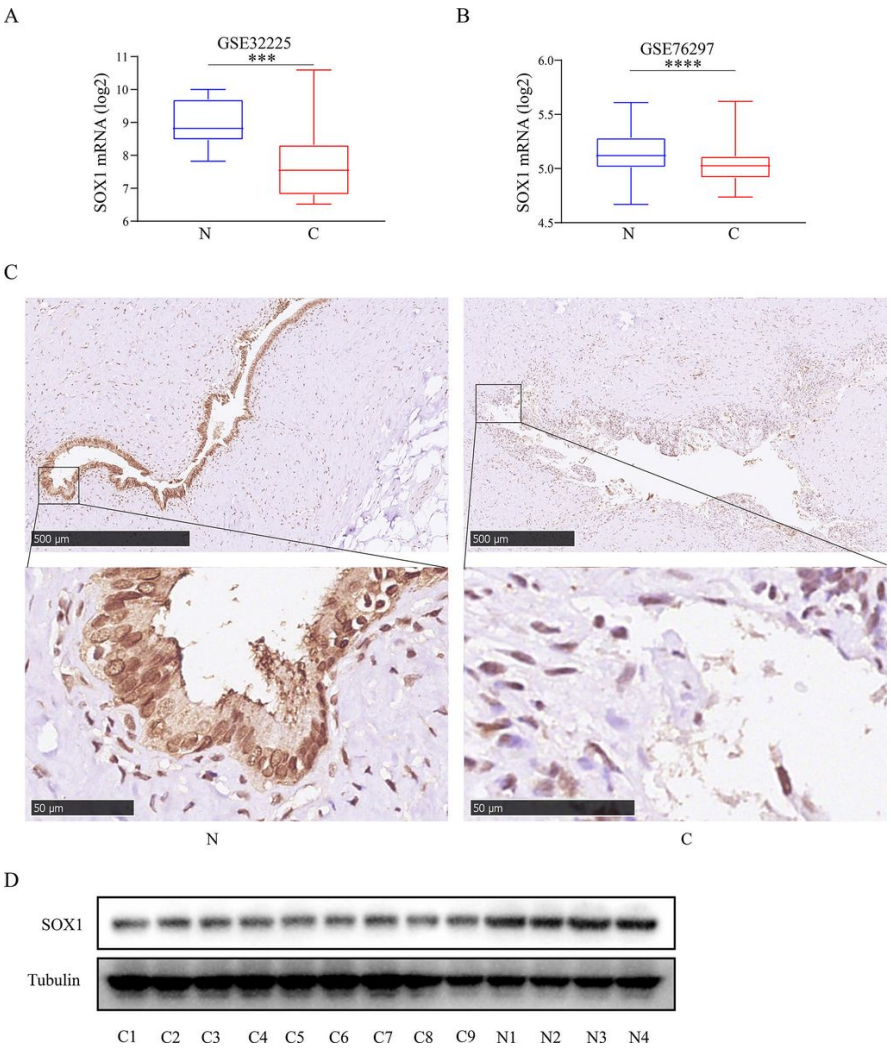

Figure 1

SOX1 is down-regulated in CCA tissues. A-B Box plots of SOX1 expression in cholangiocarcinoma (CCA) tissues compared to normal bile duct tissues. GEO databases GSE32225 (A) and GSE76297 (B) are indicated. ***p < 0.001, ****p < 0.0001. C Representative images of IHC analysis of SOX1 expression in specimens from normal bile duct tissues and CCA tissues. Upper panel shows the overall view of the entire section. Scale bars are shown. D The expression levels of SOX1 protein in 9 CCA tissues and 4 normal bile duct tissues were assessed by Western blotting. N: normal bile duct tissues; C: CCA tissues.

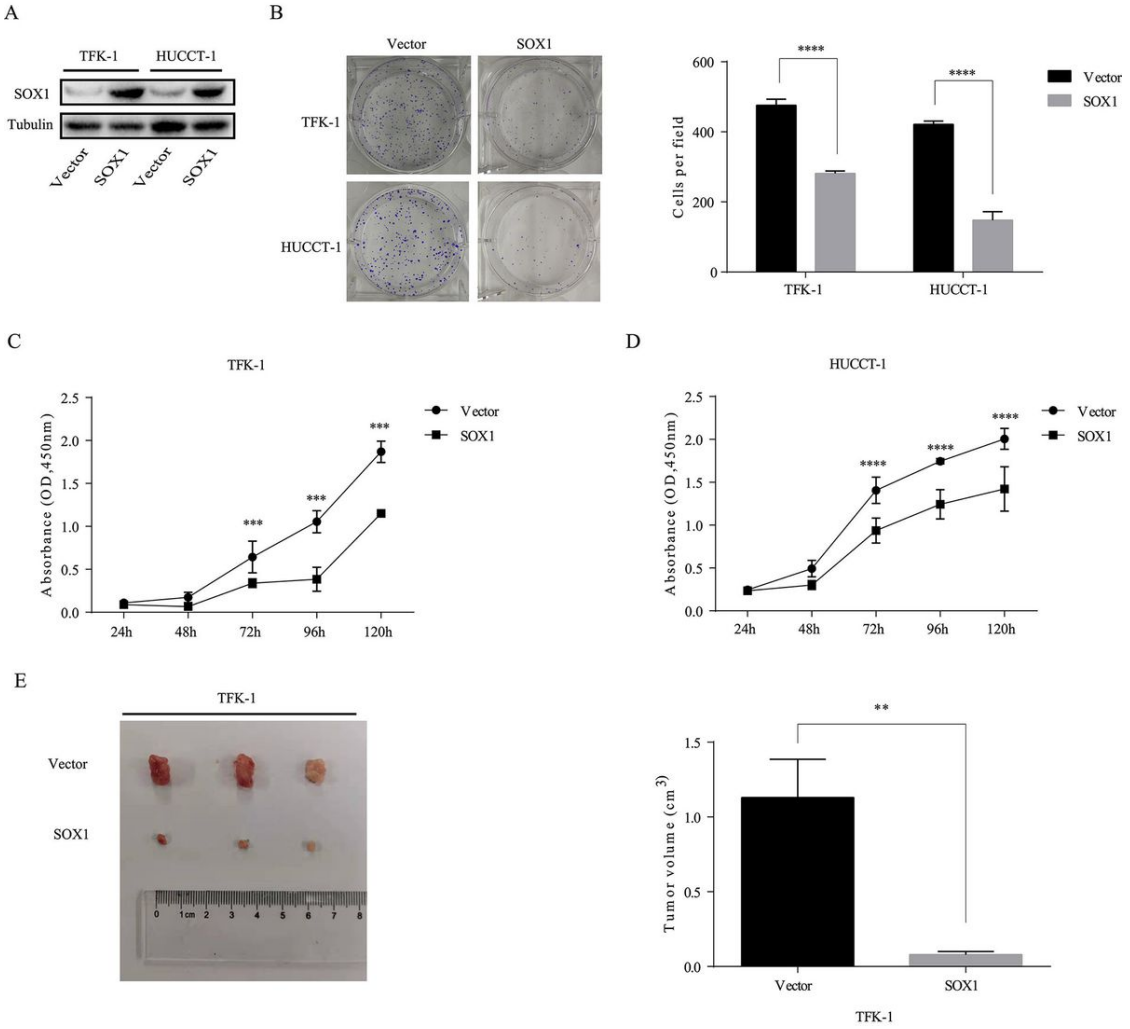

Figure 2

SOX1 inhibits CCA cells proliferation in vitro and tumor growth in vivo. A TFK-1 and HUCCT-1 cells were infected with lentiviral negative control vector (Vector) or lentiviral SOX1 (SOX1) for 48h. SOX1 protein level was examined by Western blotting. B. Representative images of colony formation assay (right panel) and analysis of colony numbers (left panel), ****p < 0.0001. C & D Cell proliferation was assessed by CCK-8 assay, ****p < 0.0001. E Right panel: Xenograft tumors at day 18 after implantation of TFK1 Vector or SOX1 cells into the right flank of nude mice are shown. Left panel: Comparison of tumor volumes between TFK1 vector and SOX1 xenograft mice, **p < 0.01.

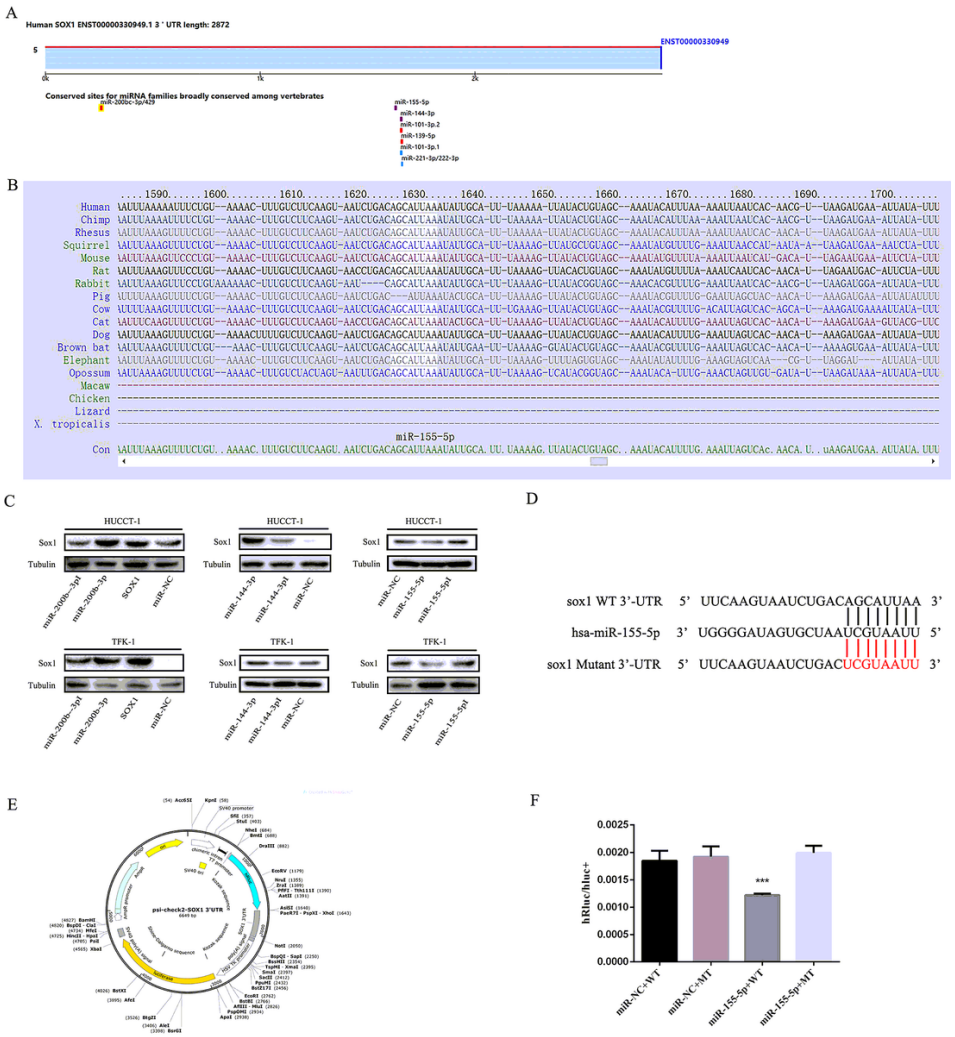

Figure 3

MicroRNA-155-5p directly targets 3'UTR of SOX1 and inhibits expression of SOX1. A Targetscan was used to search for candidate miRNAs regulating SOX1. B miR-155-5p sequences between different species. C SOX1 protein levels in CCA cells with candidate miRNAs overexpression or inhibition. D. Schematic illustration of the potential biding motifs for miR-155-5p in the wild-type (WT) 3'-UTR of SOX1 and their mutant-type (MT). E The plasmid map of psiCHECK2 which containing a SOX1 3'UTR-WT predicted binding site. F Relative activity of luciferase reporters with SOX1 3'UTR -WT and SOX1 3'UTR -MT after co-transfection with miR-155-5p mimics in HEK-293T cells. ***p < 0.001.

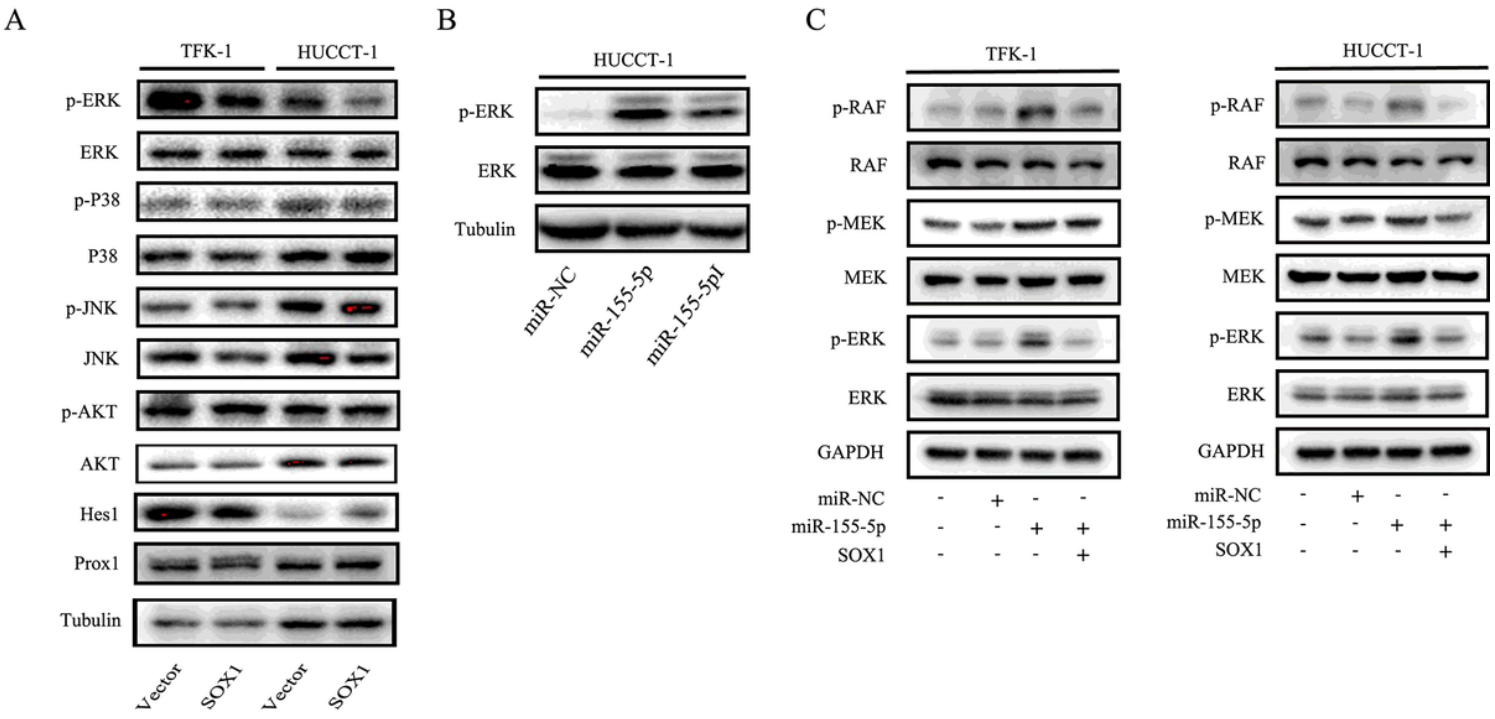

Figure 4

Mir-155-5p inhibits SOX1 to activate the Raf/MEK/ERK pathway. A Cells were infected with lentiviral negative control vector (Vector) or lentiviral SOX1 (SOX1) for 72h. SOX1, Hes1, Prox1, p-AKT, p-JNK and p-P38 were examined by western blot. B The protein levels of ERK and p-ERK in HUCCT-1 cells transfected with miR-negative control (miR-NC), miR-155-5p-mimic (miR-155-5p) and miR-155-5p-inhibitor (miR-155-5pI). C The protein levels of central members of MAPK/ERK signaling (RAF, p-RAF, MEK, p-MEK, ERK and p-ERK) were detected by western blot in TFK-1 and HUCCT-1 cells.

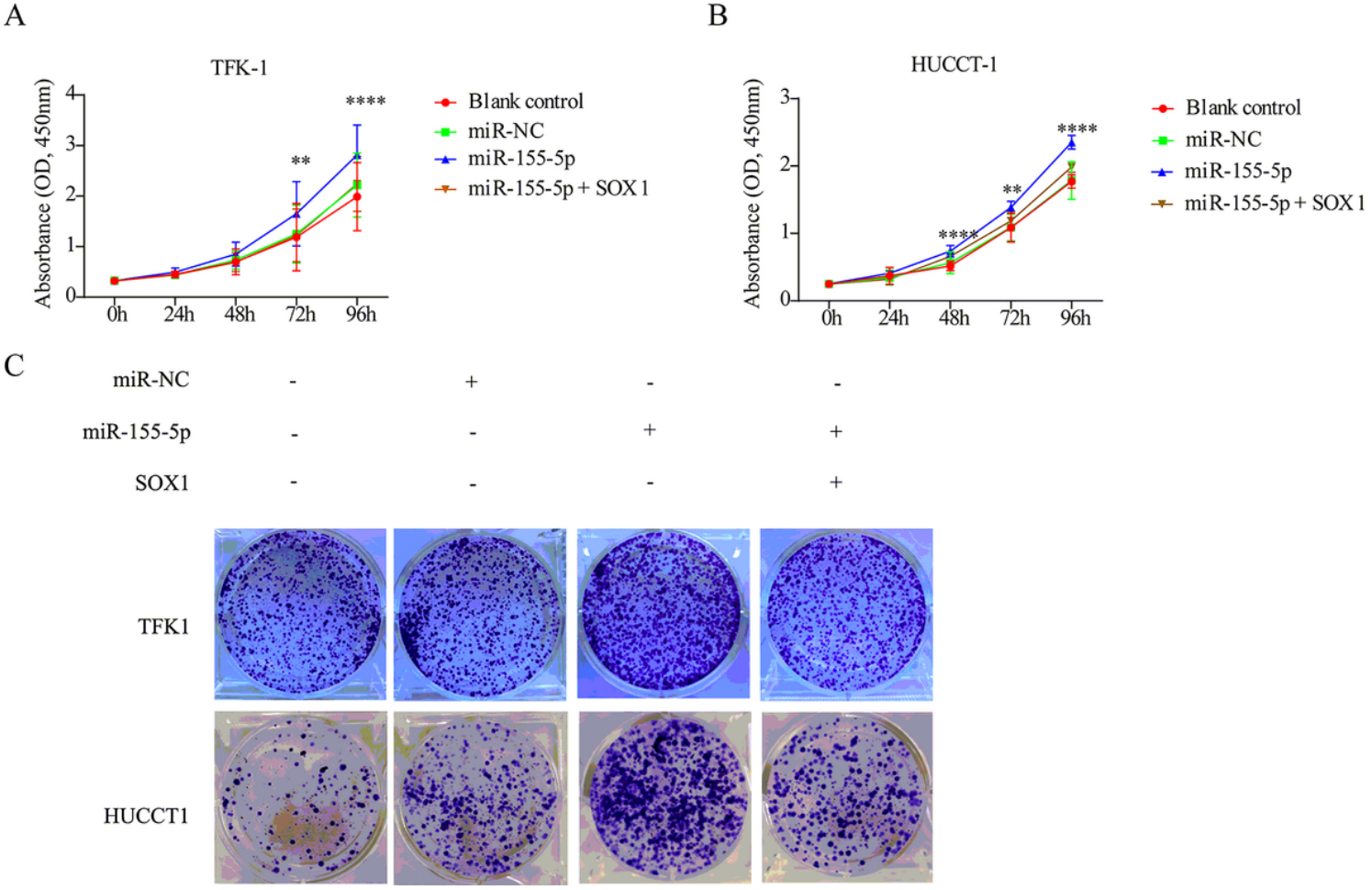

Figure 5

The miR-155-5p/SOX1 axis regulates the proliferation of CCA cells. A-B CCK-8 assays was performed to compare cell proliferation in blank control, miR-NC, miR-155-5p and miR-155-5p + SOX1 in TFK-1 and HUCCT-1 cells. All experiments were performed in triplicate, and data are presented as mean ± SD. **p < 0.01, ****p < 0.0001. C Representative images of colony formation assays of TFK-1 and HUCCT-1 cells in different groups.

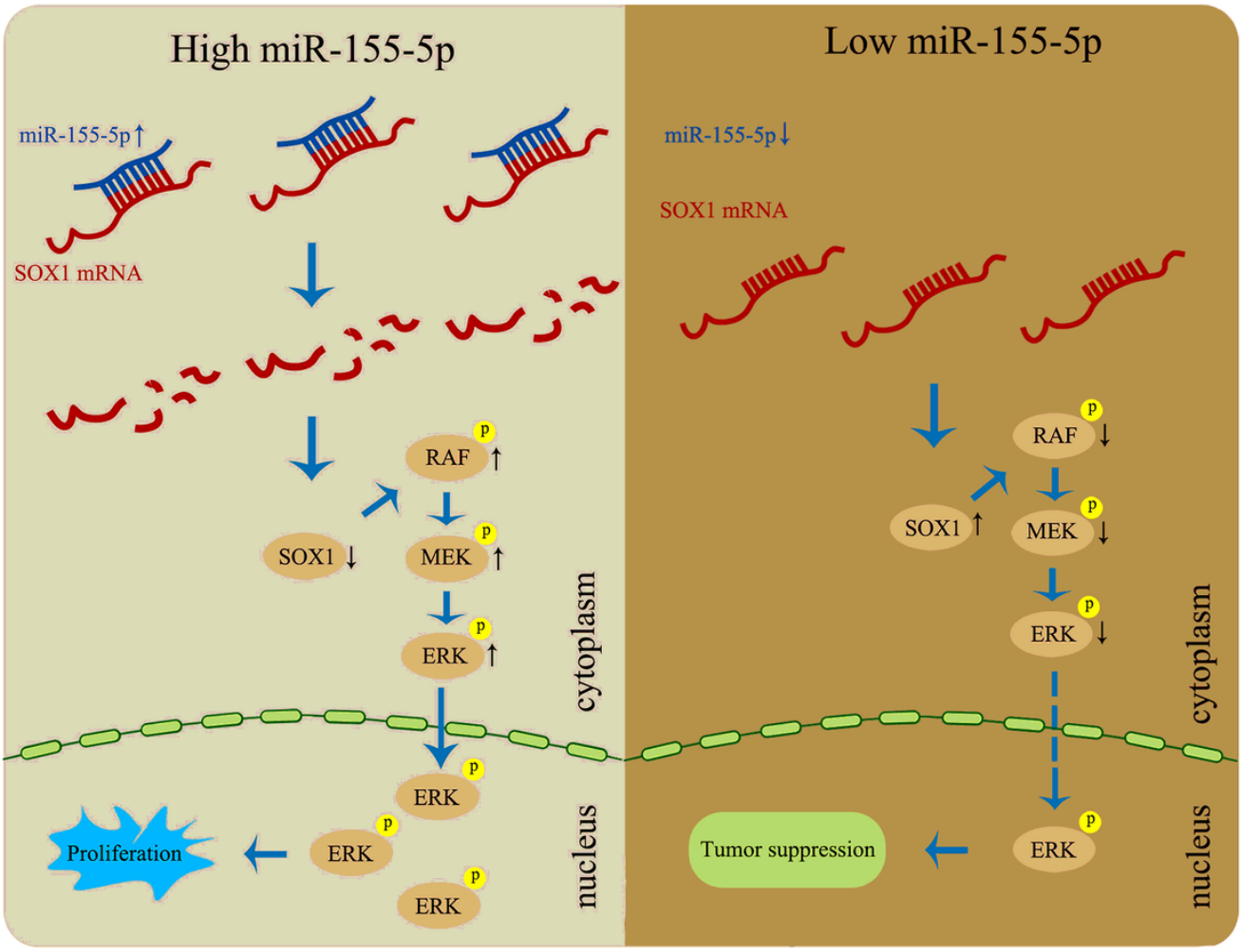

Figure 6

A Schematic model of Mir-155-5p promoting CAA progression Mir-155-5p binds to the 3'UTR region of SOX1, represses SOX1 expression, further activates the Raf/MEK/ERK pathway, thus promotes CCA progression. However, when the expression level of miR-155-5p is low, SOX1 would not be inhibited, then it would suppress the Raf/MEK/ERK pathway, block CCA progression.