

STOX2 Is the Target of Mir-30a to Increase Cell Proliferation and Metastasis in Hydatidiform Mole Through ERK, AKT and P38 Signaling Pathways

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Abstract

Background

Hydatidiform mole is a disease caused by abnormal proliferation of trophoblastic cells. MiR-30a acts as a tumor suppressor gene in most tumors and participates in the development of various cancers. But its role in hydatidiform mole is not clear.

Methods

RT-qPCR was used to verify the expression level of miR-190 and STOX2. Flow cytometry (FCM) assays were performed to detect cell cycle. CCK-8 assay, EDU assay and colony formation assay was used to detect proliferation ability. Transwell assay was used to test invasion ability. Dual-luciferase reporter assay and Western blotting were used to elucidate the potential mechanisms involved.

Result

The low expression of miR-30a promoted the ability of proliferation, migration and invasion in trophoblastic cells (JAR and HTR-8). The dual luciferase assay confirmed that STOX2 was a new target of miR-30a and resisted the effect of up-regulated miR-30a in trophoblastic cells. In addition, up-regulation of STOX2 by miR-30a could activate ERK, AKT and P38 signaling pathways. These results revealed that a new mechanism for ERK, AKT and P38 activation by miR-30a/STOX2, which could create excessive proliferation of trophoblast cells in the hydatidiform mole.

Conclusions

In this study, we found that miR-30a plays an important role in the development of HM. Taken together, these findings indicate that miR-30a may effect the malignant transformation of human trophoblastic cells by downregulating STOX2, which strengthens our understanding of miR-30a in regulating BC cell transformation.

Background

Gestational trophoblastic disease (GTD) is a group of placental trophoblast diseases characterized by abnormal proliferation[1], including hydatidiform mole, villus cancer, epithelial itch trophoblastic tumor, placental site trophoblastic tumor[2-4]. GTD can be divided into two types: benign and malignant. Benign is hydatidiform mole. The others are malignant. Choriocarcinoma can be caused by hydatidiform mole, ectopic pregnancy and abortion[5]. Hydatidiform mole (HM) is an abnormal pregnancy characterized by placental villus edema and abnormal growth of trophoblastic cells[6]. Placental edema can lead to a series of pathological phenomena, such as edema abortion (HA), partial hydatidiform mole (PHM), complete hydatidiform mole (CHM); the occurrence of hydatidiform mole accounts for 80% of GTD[7], and the incidence varies in different regions of the world[8, 9]. There are many factors that induce hydatidiform mole, such as age, race, genetic basis, spontaneous abortion and nutritional restriction[10].

The incidence of women between the ages of 21-35 is lower than those over the age of 35 or under the age of 21[11]. Compared with the average woman, women who have a history of spontaneous abortion are 2-3 times more likely to have hydatidiform mole[12]; women who have had hydatidiform moles are 10-20 times more likely to have hydatidiform moles than normal, and about 20% have the possibility of malignant transformation after resection. However, the pathogenesis of hydatidiform mole is currently unclear.

MicroRNA (miRNAs) regulates gene expression by binding to the 3'UTR region of target gene mRNA, which is a negative regulatory factor[13]. There is increasing evidence that miRNAs play an important role in the pathogenesis and progression of various tumors, regulating cell proliferation[14], cell cycle regulation[15], inflammatory response[16], cell differentiation[17], Apoptosis and metastasis[18]. It has also been reported in the literature that miRNAs also play a regulatory role during embryonic development[19]. The miRNA-518 family is a specific biomarker of placenta. miR-518b is abnormally expressed in placental tissues of preeclampsia, which not only regulates EGR1-mediated angiogenesis and migration of trophoblast cells, but also regulates the establishment of hypoxia model of early embryonic development[20]. Previous studies have shown that mir-30a-5p is located in the 6q13 region of the chromosome and is deregulated in some tumors[21]. In hepg2 and mhc97l cancer cells, overexpression of mir-30a completely blocked the activation of the k-ras/c-raf/mek/erk pathway. These findings suggest that mir-30a may play a role in the growth, apoptosis and metastasis of hepatoma cells by regulating the k-ras/c-raf/mek/erk signaling pathway, which may become a targeted biomarker for liver cancer treatment[22]. MiR-30a is overexpressed in the placenta of patients with eclampsia. The possible mechanism is to influence the invasion and apoptosis of trophoblast cells by targeting IGF-1[23].

STOX2 is a winged helix domain, on chromosome 4q35, near the chromosomal region associated with preeclampsia, it is a transcription factor involved in trophoblast differentiation and the most important collateral of STOX1 [24, 25]. The abnormal expression of STOX2 in neural crest stem cells and lung cells of the offspring of asthmatic inflammatory model mice was analyzed by transcription expression[26]; compared with the mice without pregnancy, the inflammatory response of stox2 in pregnant mice to air pollutants was increased[27]. Melanoma suppressor protein (MIA) affects the expression of STOX2 by a paracrine manner, promotes the proliferation and metastasis of oral squamous cell carcinoma, and finds that STOX2 is combined with anticancer drugs such as paclitaxel, cisplatin or 5-FU, which can reduce the drug resistance of cancer cells, provide a new treatment for diseases[28]. We compared the expression levels of STOX1 and STOX2 in decidual tissue with pregnancy with pre-eclampsia and/or fetal growth restriction (FGR), and found that STOX1 did not observe differential gene expression in any case group, while the expression of STOX2 in decidua of pregnancy with preeclampsia and FGR was significantly lower than that in the control group[29].

In the present study, the results of previous experiments showed that miR-30a was lowly expressed in hydatidiform mole tissue. Combined with laboratory database and biological software prediction, STOX2 is a possible target of miR-30a. We found that miR-30a active the AKT, ERK, P38 signaling pathways affects the development of hydatidiform moles by regulating STOX2.

Materials And Methods

Cell culture and transfection

Human trophoblast cell line JAR and the human cervical cancer cell line HeLa were purchased from the American Type Culture Collection (Manassas, VA, USA). HTR-8/SVneo cell was obtained from the Animal Institute of the Chinese Academy of Sciences. And cells were maintained in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, USA) containing 10% fetal bovine serum (ScienCell, USA), 1% penicillin-streptomycin solution (Thermo Fisher Scientific, USA). Cultures were maintained in the cell incubator with a humidified atmosphere of 5% CO₂ at 37 °C

MiR-30a mimics and mimics negative control, miR-30a inhibitor and inhibitor negative control were purchased from Guangzhou RiboBio Co.,LTD. r. Small interfering RNA (siRNA) for STOX2 and negative control were produced by Shanghai GenePharma (Shanghai, China). The si-STOX2 sequence was 5'-AUGGGAGACAUACUGAUGGTT -3' and the si-NC sequence was 5'-ACGUGACACGUUCGGAGAATT -3'. STOX2 DNA and corresponding negative control were composed by GeneCopoeia Inc. The cells were seeded in 6-well plates after 70%-80% confluence, the transfection was performed by Lipofectamine® 2000 reagent according to the manufacturer's descriptions.

RNA extraction and Quantitative RT-PCR

The total RNAs were extracted from JAR and HTR-8 cells by using TRIzol reagent. cDNA is synthesized using TransScript All-in-one First-Strand Cdna Synthesis SuperMix for qPCR kit (One-Step gDNA Removal) (TransGen, Beijing, China) according to the manufacturer's specifications. qPCR was performed by using a TransStart Top Green qPCR SuperMix (TransGen, Beijing, China) with analysis in an ABI 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The qPCR conditions were 94°C for 30 sec followed by 40 cycles of denaturation at 94°C for 5 sec and annealing/elongation at 60°C for 30 sec. U6 was used as the internal reference for miR-30a, and GAPDH was performed as the control for STOX2 expression. The PCR primer was shown as follows: STOX2 forward: 5'-AGCCTGTCCCTCCTCAAATCTCA-3', reverse: 5'-CTCTGTGTTCTTGTGGCCCT-3'; GAPDH forward: 5'-GTGAAGGTCGGAGTCAACG-3', reverse: 5'-TGAGGTCAATGAAGGGGTC -3'; the data was calculated using $2^{-\Delta\Delta ct}$ method. All experiments were performed triplicate samples.

CCK-8 assay

Transfected Cells were seeded 96-well plates in 200 µl medium at the density of 5000 per well and cultured for 24, 48, 72h, cck-8 (Dojindo, Tokyo, Japan) was added into the each well according the manufacturer's specifications. After 4h in 37°C incubation, the absorbance at 450 nm were detected using a microplate reader(BioTek, USA).

Colony formation assay

After Transfected 48h, 3×10^3 Cells were seeded 6-well plates and incubated for 15 days. The colonies were fixed with methanol for 30 min and stained with 1% crystal violet for 15 min. Cells number was counted by using Image J.

EdU assay

For transfected cells, EdU reagent (Beyotime, Shanghai, China) was added into 96-well plates and cultured 2h in 37°C incubator. Next, Cells were fixed with 4% paraformaldehyde for 30 minutes and 0.1% Triton-X 100 for 15 minutes, and incubated with Azide-488 for 30 minutes at room temperature in the dark room. Images were taken under inverted microscope (Olympus, Japan).

Transwell assay

8- μ m pore size 24-well Transwell plates (Corning, USA) were used to evaluate the Migratory and invasive potential of JAR and HTR-8 cells. For migration assay, transfected cells were collected and resuspended in 1 ml serum-free RPMI-1640 medium, 5×10^4 cells were added to the the upper chambers, RPMI-1640 medium with 10% FBS was seeded into the lower chambers. After 24h in incubator with 5% CO₂ 37°C, the migratory cells were fixed with methanol for 30 min and stained with 0.2% crystal violet for 30 min. For invasion assay, the upper chambers of transwell were pre-coated with Matrigel (BD Biosciences, USA), 5×10^4 transfected cell were seeded into the upper chambers with serum-free RPMI-1640 medium, after incubated 30h, the cells which not invasive were wiped off with a cotton swab, and the invasive cells were fixed with methanol for 30 min and stained with 0.2% crystal violet for 30 min. All migratory and invasive chambers were counted under a light microscope (Olympus, Japan).

Western blot assay

After the cells were transfected for 48h, total protein was cleaved using ProteinExt® Mammalian Total Protein Extraction Kit (TransGen, Beijing, China), and the protein was determined by the BCA method (TransGen, Beijing, China). 30 μ g or 60 μ g protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 10%) and transferred onto a nitrocellulose membrane (Millipore, Bedford, MA, USA) using cold transfer buffer. The membrane was stained with Ponceau S (Beyotime, Shanghai, China) and washed with TBST. 5% non-fat milk was used to block the membrane for 2h at room temperature. The membrane was incubated with the primary antibody overnight 4°C. The list of primary antibodies is as follows: STOX2 (1:1000, Abcam), ERK (1:1000, Beyotime), p-ERK (1:500, Beyotime), AKT (1:1000, Beyotime), p-AKT (1:500, abcam), P38 (1:1000, Elabscience, wuhan, China), p-P38 (1:500, Elabscience, wuhan, China), GAPDH (1:4000, proteintech). Next day, the membrane was washed and the secondary antibody was incubated at room temperature for 1 h. Blots were then developed by chemiluminescence with Pierce ECL kits (Thermo scientific, USA). The data was used to analysis by Image J software.

Luciferase activity assay

The miRNA prediction website (miRBase, TargetScan, PicTar) was assessed the binding site of miR-30a and STOX2 3'UTR. The 3'-UTR of STOX2 were synthesised by PCR and cloned into the XhoI site downstream of Renilla luciferase genes in the PmiR report vector (Promega, USA), the 3'UTR of STOX2 primer forward: 5'-CCGCTCGAGCGGCAGATCTTCTGTCTCATTTCGATAACA-3'

Reverse: 5'-CCGCTCGAGCGGCAGATCTTCTGTCTCATTTCGATAACA-3'. WT or Mut seed sequences of 3'UTR of STOX2 were constructed onto the PmiR report vector. HeLa cells were seeded into 12-well plates and cotransfected with miR-30a mimics or negative control and WT or Mut vector, after incubation of 24h, the Dual-Luciferase® Reporter Assay was implemented with the manufacturer's manual (Promega, USA). The luciferase activities were measured by a Fluorescence/Multi-Detection Microplate Reader (BioTek, USA)

Cell cycle analysis

48h after transient transfection, the cells was collected and washed with PBS two times, then fixed with cool 70% ethanol overnight at 4°C. The fixed cells were stained with PI/RNase solution (Sungene, Tianjin, China) for 30 min at room temperature and analyzed using a FACS Calibur (BD Biosciences, USA). The percentage of cells in each period was analysed by ModFit software.

Immunohistochemistry (IHC)

Tissue sections were dewaxed in xylene and dehydrated gradient ethanol, the activity of endogenous peroxidase was activated and blocked with 3% H₂O₂ for 20 min in the dark room. Goat serum was added on the tissues by using a dropwise way for 20 min at room temperature. Then, the sections were incubated with the following primary antibodies overnight at 4°C:STOX2 (1:100, Abcam), AKT (1:70, Beyotime), ERK (1:100, Beyotime), p-ERK (1:50, Beyotime), p-AKT (1:70, abcam), P38 (1:100, Elabscience, wuhan,China), p-P38 (1:50, Elabscience, wuhan,China). Next, the second antibody and horseradish peroxidase streptavidin were maintained for 30 min at 37°C, respectively. The sections were stained by DAB coloration (OriGene Technologies, Beijing, China) and hematoxylin stain (KeyGEN BioTECH, Jiangsu, China). Tissues were imaged with a light microscope.

Hematoxylin and eosin (HE) staining

The Hydatidiform mole and normal placenta tissues were fixed in 4% formaldehyde and embedded in paraffin for Hematoxylin and eosin (HE) staining. The slices were stained with hematoxylin for 20 min and eosin for 30 s-1 min. Finally, the slices were analyzed under a light microscope.

Statistical Analysis

All data were presented as mean ± SD and analysed by using GraphPad Prism 6.0 software. All experiment was repeated three times independently. Significance of differences between the two groups was assessed via One-way ANOVA and P value <0.05 was considered statistically significant.

Results

Up-regulation of miR-30a inhibited trophoblastic cells of proliferation, cell cycle and metastasis

In previous studies, we demonstrated that the expression level of miR-30a was lower in hydatidiform moles which can be seen in our earlier article [49]. However, the regulatory mechanism of miR-30a in hydatidiform mole is still unclear. In this article, we will explore the mechanism of miR-30a in hydatidiform mole disease at the cellular level. The transfection efficiency was detected by treating miR-30a mimics/negative control and miR-30a inhibitor/negative control in HTR-8 cells (Figure 1 A). CCK-8 assay was performed to discover the effect of miR-30a on the proliferation of HTR-8 cells, the results showed that the cell vitality of HTR-8 cells was weakened by up-regulating miR-30a. Conversely, the suppression of miR-30a was increased cell proliferation (Figure 1 B). The colony formation ability was reduced by transfecting miR-30a mimics (Figure 1 C and Figure 1S A). Compared with transfected negative control, the fluorescence activity of cells transfected miR-30a mimics were decreased, suggesting that cell proliferation was slow (Figure 1 D). Furthermore, we analyzed that down-regulated miR-30a could enhance the cell rate in S stage, promoting the growth of trophoblastic cells (Figure 1 E and Figure 1SB). To investigate the effect of migration and invasion of HTR-8 cells, we operated transwell assay. Compared with transfected negative control, miR-30a transfected miR-30a mimics impaired the ability of migration and invasion, and stronger ability of metastasis was observed by down-regulating miR-30a (Figure 1 F and G). In conclusion, the data demonstrated that miR-30a restrained cell viability, cell cycle and metastasis in trophoblastic cells.

The effect of different expression levels of stox2 on the proliferation and metastasis of trophoblastic cells

To investigate the effect of STOX2 on the proliferation and metastasis of trophoblast cells, firstly, we tested the transfection efficiency by transfecting STOX2 siRNA or STOX2 cDNA using Real-time PCR (Figure 2 A and C). We performed CCK-8, colony formation assay and EdU assay in JAR and HTR-8 cells. In CCK-8 experiment, the results showed that up-regulation of STOX2 significantly promoted the cell proliferation ability; compared with the control group, the proliferation ability of cells was significantly weakened following down-regulated STOX2 (Figure 2 B and D). In colony formation assay, the ability of cells transfected with stox2 cDNA to form colonies increased, but the ability of cells interfering with STOX2 siRNA group to form colonies decreased significantly (Figure 2 E and F); Similarly, EdU assay found that cell replication and the fluorescence activity were enhanced after transfecting up-regulated STOX2; otherwise the fluorescence activity decreased (Figure 2 G and H).

Subsequently, Transwell assay found that overexpression of STOX2 enhanced cell migration and invasion, and the ability of metastasis was impaired by reducing the expression of STOX2 (Figure 2 I and J, K and L). These results indicated that up-regulation of stox2 could promote cell proliferation, migration and invasion.

miR-30a targets the 3'-UTR of STOX2 mRNA and is low in hydatidiform mole.

To explore the effects of miR-30a on trophoblast cell proliferation, migration and invasion, STOX2 was predicted to be a candidate target gene for miR-30a through the biological software (miRBase, TargetScan, PicTar). As shown in Fig. 3A, it was found that miR-30a had a binding site with the 3'UTR region of STOX2. Subsequently, we cloned WT and Mut 3'UTR of STOX2 into a luciferase reporter vector. It constructs containing the renilla luciferase gene fused to STOX2 3'UTR sequence and the firefly luciferase for normalization. These vectors were transfected in HeLa cells with miR-30a mimics or Negative. As expected, compared with co-transfecting WT STOX2 3'UTR PmiR and negative, the luciferase reporter activity in cells was reduced co-transfected WT STOX2 3'UTR PmiR and miR-30a mimics; in the same way, we found that the luciferase reporter activity was no changed when we co-transfected Mut STOX2 3'UTR PmiR and miR-30a mimics or negative (Figure 3 B). Further evidence of the impact of miR-30a on STOX2 was measured by the expression of STOX2 by western blot. The protein level of STOX2 was reduced in JAR and HTR-8 cells by transfecting with miR-30a mimics, when we inhibited the expression of miR-30a, we found that STOX2 protein was increased (Figure 3 C and D). In our previous research, we detected that miR-30a was lower in hydatidiform mole tissues than normal placenta, but now we also found that the expression of STOX2 was high in hydatidiform mole tissues (Figure 3 E). These results confirmed our prediction that STOX2 is a new target of miR-30a and miR-30a might be participated in the pathogenesis of hydatidiform mole by targeting STOX2.

STOX2 mitigates miR-30a stimulation of proliferation and metastasis in trophoblastic cells

To investigate the functions of STOX2 and miR-30a in trophoblastic cells, in previous research, we found that increasing miR-30a could inhibit the proliferation and metastasis abilities in JAR and HTR-8 cells. We performed the CCK-8 (Figure 4 A and B, Figure 5 A and B) colony formation assay (Figure 4 C and D, Figure 5 C and D) EdU assay (Figure 4 A and B, Figure 5 A and B) to explore the proliferation of cells. The results showed that over-expression of miR-30a and STOX2 could reinforce the proliferation of cells compared with miR-30a mimics group. Conversely, when we transfected the down-expression of cells, the results clearly demonstrated the opposite trends. For the migration and invasion of cells, transwell assay revealed that enhanced expression of STOX2 impaired the ability of miR-30a mimics to decrease the trophoblastic cells metastasis (Figure 4 G and I, Figure 5 G and I). Compared with miR-30a inhibitor group, co-transfection with STOX2 siRNA and miR-30a inhibitor group was also blocked migration and invasion (Figure 4 H and J, Figure 5 H and J). These data suggest that STOX2 alleviate the proliferation and metastasis of JAR and HTR-8 cells.

MiR-30a Induces STOX2 expression and through ERK 1/2, AKT, and p38 Signaling Pathway in trophoblastic cells

We found that miR-30a and STOX2 mediated trophoblastic cells proliferation and metastasis in vitro. To study the molecules mechanisms for miR-30a and STOX2 induced inhibition of growth, invasiveness and migration of JAR and HTR-8. With transfected miR-30a mimics, the phosphorylation of AKT, ERK and P38 was decreased and the expression of AKT, ERK and P38 was not changed. Conversely, up-regulation of STOX2 increased the expression of p-AKT, p-ERK, p-P38. Co-treatment with miR-30a mimics and STOX2

cDNA could further partially strengthened the expression of p-AKT/p-ERK/p-P38, and the total protein of AKT/ERK/P38 was not changes (Figure6 A and C). In the same way, similar levels of AKT/ERK and P38 protein expression were observed in the miR-30a inhibitor and STOX2 siRNA-transfected group, miR-30a inhibitor group, STOX2 siRNA group and the NC-transfected group. Transfected STOX2 siRNA weaken the protein level of p-AKT/p-ERK/p-P38 on miR-30a inhibitor in JAR and HTR-8 cells (Figure6 B and D). These results suggested that miR-30a regulated AKT/p-AKT, ERK/p-ERK, P38/p-P38 signaling pathway by inhibiting cell proliferation, metastases through targeting STOX2.

Discussion

Hydatidiform mole is one of the most common Gestational trophoblastic diseases, with a prevalence of 1:1000 in North America and Europe, but is more common in South America and Asia. Due to differences in histology, genetics, and clinical features, hydatidiform moles can be divided into complete moles (CHM) and partial moles (PHM)[30, 31]. Ultrasound is the main method for clinical detection of hydatidiform mole, but it is also determined by the age of the fetus. In the world, hydatidiform mole is diagnosed earlier, but in developing countries, there are still patients diagnosed in the early pregnancy with complications[32]. Although hydatidiform moles are benign, there is still a high possibility of developing malignant trophoblastic tumors. The clinical treatment is curettage and hysterectomy. In the later stage, the content of hCG should be detected to prevent recurrence. These not only cause irreparable physical damage to patients, but also cause huge psychological burden. Therefore, new treatment methods are urgently needed.

miRNAs regulate many genes and are involved in the development of many tumors[33, 34]. Mir-30a plays a key role in many types of human cancers, mainly anti-cancer effects, non-small cell carcinoma[35], breast tumor[36], renal cell carcinoma[37], colorectal cancer[38]. miR-30a prevents DNA replication and leads to DNA degradation by targeting RPA1, which induces P53 expression, and triggers S-phase checkpoints, prevents cell cycle progression, ultimately leads to cancer cell death[39]. miR-30a regulates Eya2 to mediate the G1/S cell cycle and the expression of related cyclins[40]. In gallbladder cancer, miR-30a could directly target e2f7 to regulate EMT and metastasis, and participated in cancer progression [41]. The expression of miR-30a-3p was significantly increased in the preeclamptic placenta and regulated trophoblast invasion and apoptosis by targeting IGF-1[23]. In the present study, we have found that down-regulated miR-30a was boosted the ability of proliferation, migration, invasion in trophoblastic cells, and was lower expression in hydatidiform mole tissues than normal placenta, which suggests that miR-30a was involved in the development of hydatidiform mole.

Storkhead box 2 (STOX2) is considered to be the only collateral family of STOX1, but as far as we know, its function is still unclear. However, a large number of studies on STOX1 have reported that the polyploidy defect before incomplete invasion of extravillous trophoblast caused by STOX1 dysfunction seems to be the center of pre-eclampsia, activating PI3K/AKT/FOX signaling pathway[24]. It has been reported in the literature that STOX1A regulates cell cycle by binding to CCNB1 to regulate mitosis[42]. An increase in the level of insulin-like growth factor 1 (IGF1) leads to an increase in the expression of STOX1

in the extravillous trophoblast through the MAPK pathway, thereby identifying a new signaling cascade involved in maternal-fetal communication[43]. Homologous genes have similar biological functions. The RNA encoded by intron 3 of STOX2 transcription can affect the alternative splicing of host genes in placental cells. STOX2-IT3-lncRNA affects genes involved in trophoblast differentiation and invasion, thus affecting the pathogenesis of eclampsia[44]. In this study, we found that STOX2 was not only reduced cell proliferation and metastasis, but also was highly in hydatidiform mole tissues, which suggests that stox2 may induce hydatidiform mole.

It was well known that the biological role of miRNAs was achieved by regulating downstream target genes. Our results revealed that STOX2 was the directly target gene of miR-30a. Moreover, we found that STOX2 was reduced by up-regulating miR-30a in trophoblastic cells. And we also detected that down-regulated STOX2 could impair the ability of proliferation and metastasis in trophoblastic cells by transfected miR-30a inhibitor. However, the molecular mechanism by miR-30 regulates the biological role of STOX2 is still not very clear. ERK, AKT, and P38 signaling pathways are important for tumor proliferation and metastasis, and involved in the pathogenesis of various cancers. Some studies have shown that miR-30a-5p targeting NeuroD1 could improve inflammatory response and oxidative stress through MAPK / ERK signaling pathway in case of spinal cord injury.[45]. miR-30a released by p53 r273h mutation can inhibit the expression of IGF-1R, which leads to the activation of igf-1r-akt signal cascade in tumor cells [46]. Mir-30a is significantly down-regulated in highly metastatic colorectal cancer cell lines and metastatic tissues, and its mechanism mediates Akt/mTOR signaling pathway by targeting PIK3CD expression, regulating the metastasis of cancer cells [47]. In addition, mir-30-5p-tcf21-mapk/p38 signaling pathway may be a potential biomarker or therapeutic target of atherosclerosis [48]. Interestingly, in our study, we demonstrated that overexpression of miR-30a inhibited the phosphorylation of ERK, AKT, P38, whereas up-regulated STOX2 ameliorated this suppressive effect. These data further confirmed that the aggressive proliferation potential of hydatidiform mole was caused by the low expression of miR-30a, at least in this part, by regulating STOX2 through activating ERK, AKT, P38 signaling pathways. Further research of the specific molecular mechanism was required on this process.

In the summary, we recognized STOX2 high-regulated in hydatidiform mole. miR-30a was able to suppress the proliferation and invasion ability of trophoblastic cells through targeting STOX2-mediated AKT, ERK and P38 signaling pathways. Our findings may offer insight into a new mechanism of hydatidiform mole. MiR-30a and STOX2 could be a biomarker and therapeutic target for hydatidiform mole in the future time.

Conclusions

In the summary, we recognized STOX2 high-regulated in hydatidiform mole. miR-30a was able to suppress the proliferation and invasion ability of trophoblastic cells through targeting STOX2-mediated AKT, ERK and P38 signaling pathways. Our findings may offer insight into a new mechanism of hydatidiform mole. MiR-30a and STOX2 could be a biomarker and therapeutic target for hydatidiform mole in the future time.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

Neither this paper nor any similar paper has been or will be submitted to or published in any other scientific journal. All authors are aware and agree to the content of the paper and to their being listed as an author on the manuscript.

There is no conflict of interest or competing financial interests for all authors

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Authors' contributions

Zhenzhen Guo, Chenyu Zhu and Zhen Li performed the experiments. Youhui Wang and Lu Wang analyzed the data. Na Zou and Dong Li collected tissues. Jianhui Fan, Yuefei Xu wrote and revised the manuscript. Linlin Sui and Ying Kong designed the experiments. All authors reviewed the final version of the manuscript.

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Figures

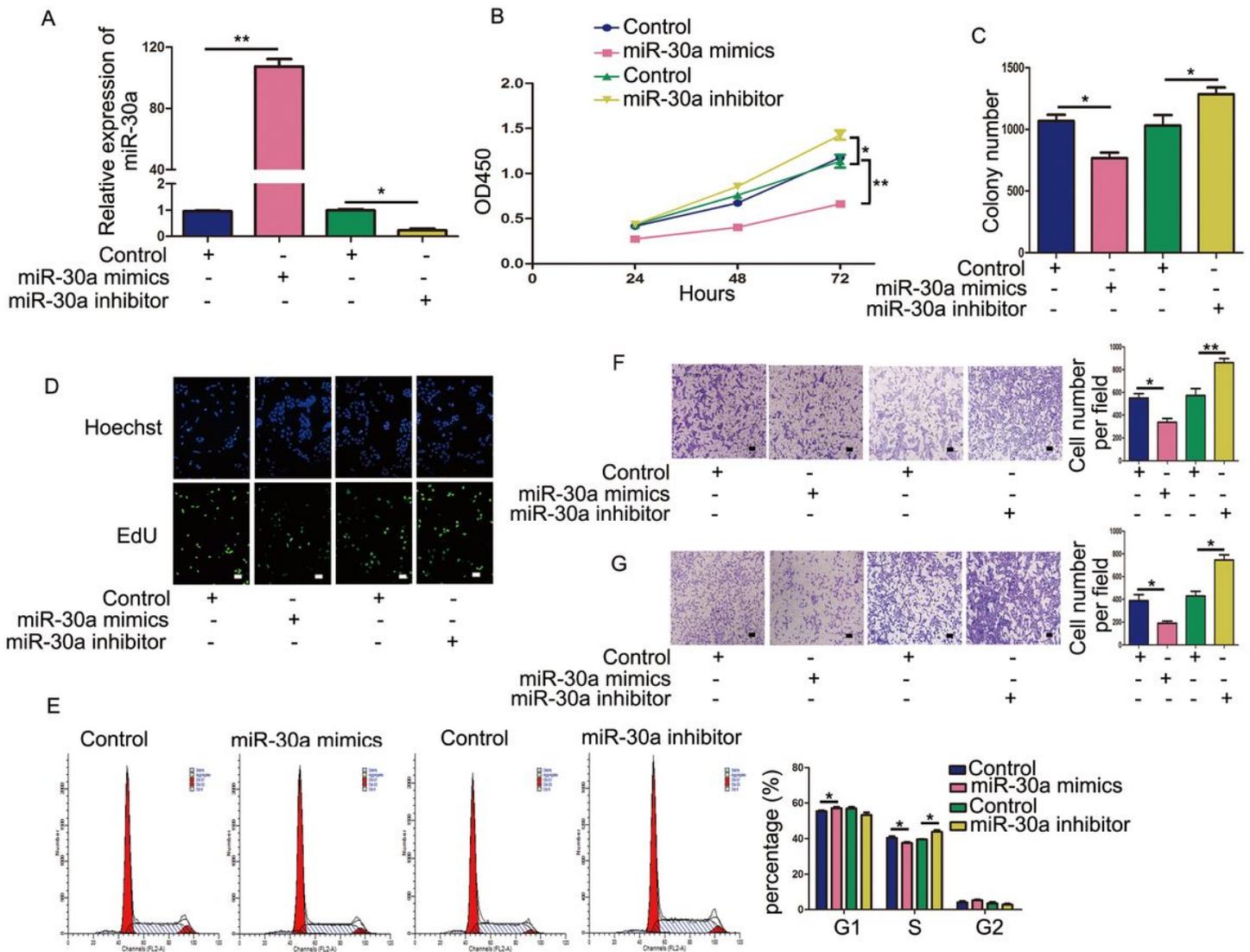


Figure 1

Up-regulation of miR-30a inhibited the proliferation, cell cycle and metastasis of HTR-8 cells. (A) The level of miR-30a was detected by qRT-PCR. (B) CCK-8 assay was used to test the proliferation of HTR-8 by transfecting miR-30a mimics/negative control or miR-30a inhibitor/negative control. (C) The cell vitalities were determined by colony formation assay. (D) EdU assay was detected the growth ability of HTR-8 under the different levels of miR-30a. (E) Cell cycle was analyzed by flow cytometry. The migration (F) and invasion (G) of HTR-8 cells was analyzed by Transwell assay. Bar=100µm. *P<0.05, **P<0.01.

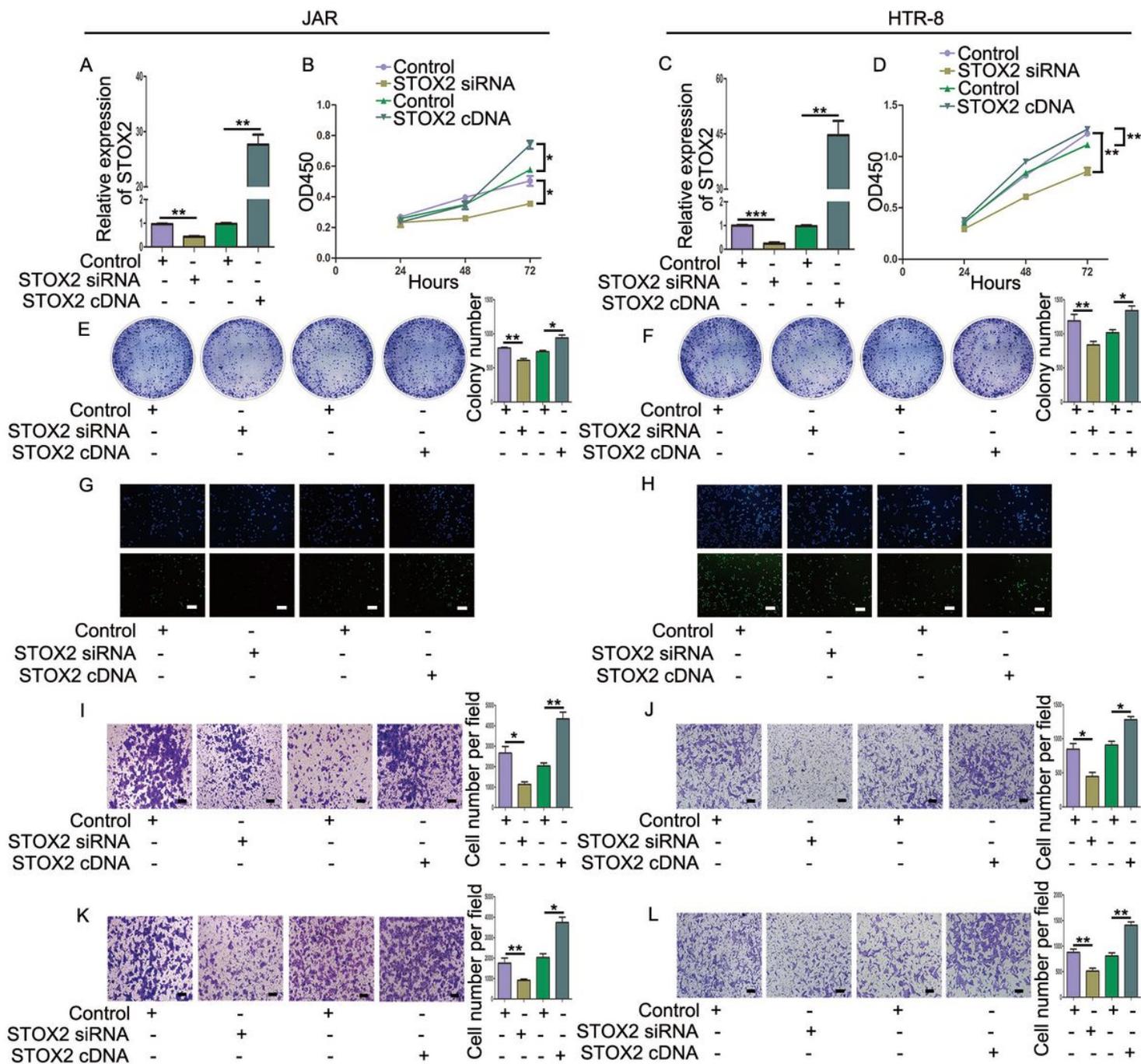


Figure 2

The effect of different expression levels of *stox2* on the proliferation and metastasis of trophoblastic cells. (A and C) The *STOX2* relative expression was detected RT-qPCR by transfected *STOX2* siRNA/negative control or *STOX2* cDNA/negative control in JAR and HTR-8 cells. (B and D) The effect of *STOX2* on JAR and HTR-8 cells proliferation was showed in each group. (E and F) Colony formation assay was detected with the different level of *STOX2*. (G and H) Cell growth distribution was tested after transfected *STOX2* siRNA or *STOX2* cDNA into JAR and HTR-8 cells. Transwell assay was used to find the ability of migration (I and J) and invasion (K and L). Bar=100µm. *P<0.05, **P<0.01.

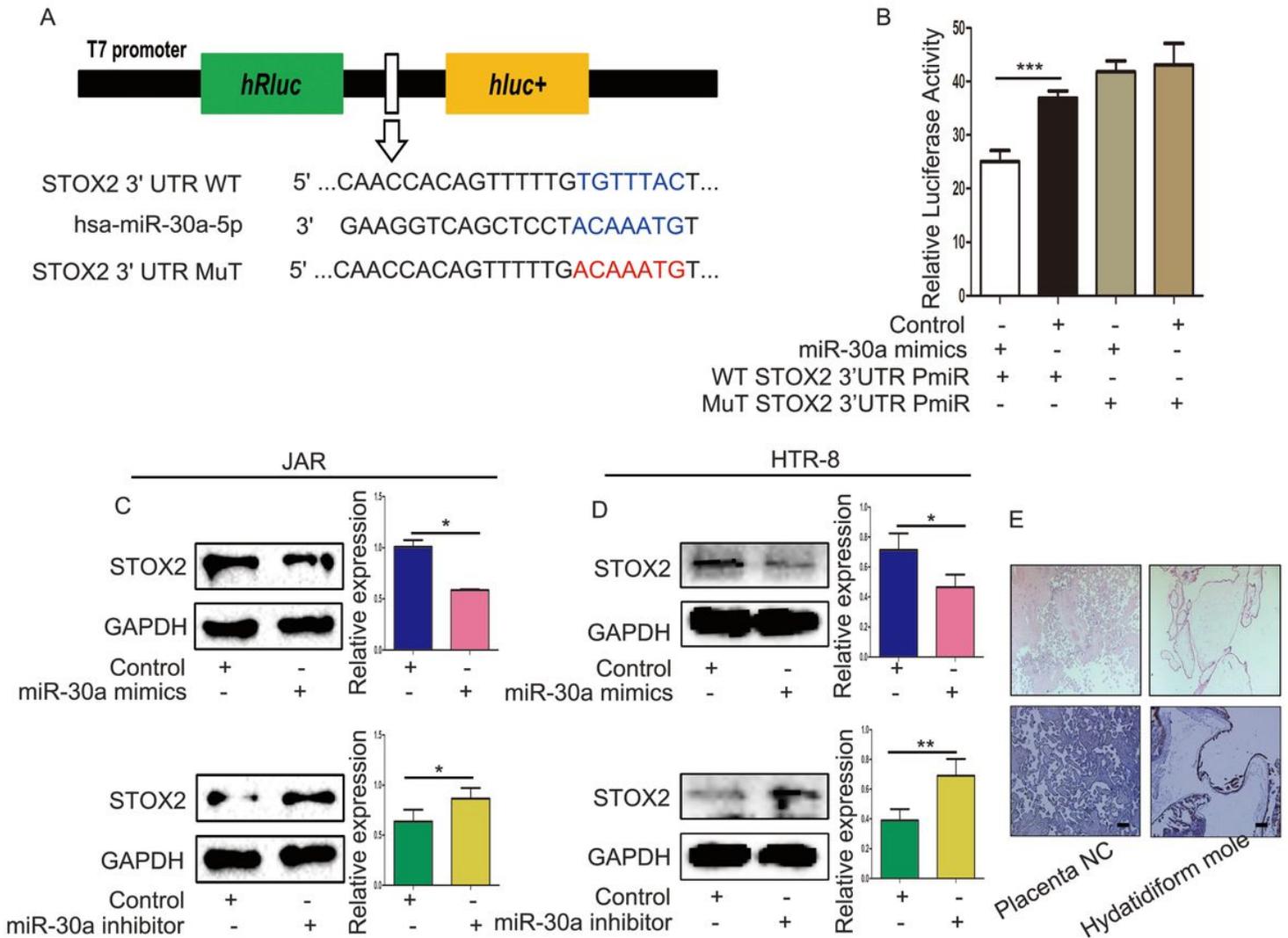


Figure 3

miR-30a targets the 3'-UTR of STOX2 mRNA and is low in hydatidiform mole. (A) miR-30a-5p binds to the sequence of the STOX2 3'-UTR region, and mutating the binding site, construction of wild-type and mutant vectors, respectively. (B) The HeLa cells were transfected with wild or mutant report Vector with miR-30a mimics to determine the luciferase activity. (C and D) Western Blot assay was detected the protein expression levels of STOX2 after regulating of miR-30a. (E) IHC-P was found the expression of STOX2 in hydatidiform mole tissues and normal placenta. Bar=100 μ m. *P<0.05 or **P<0.01, ***P<0.001.

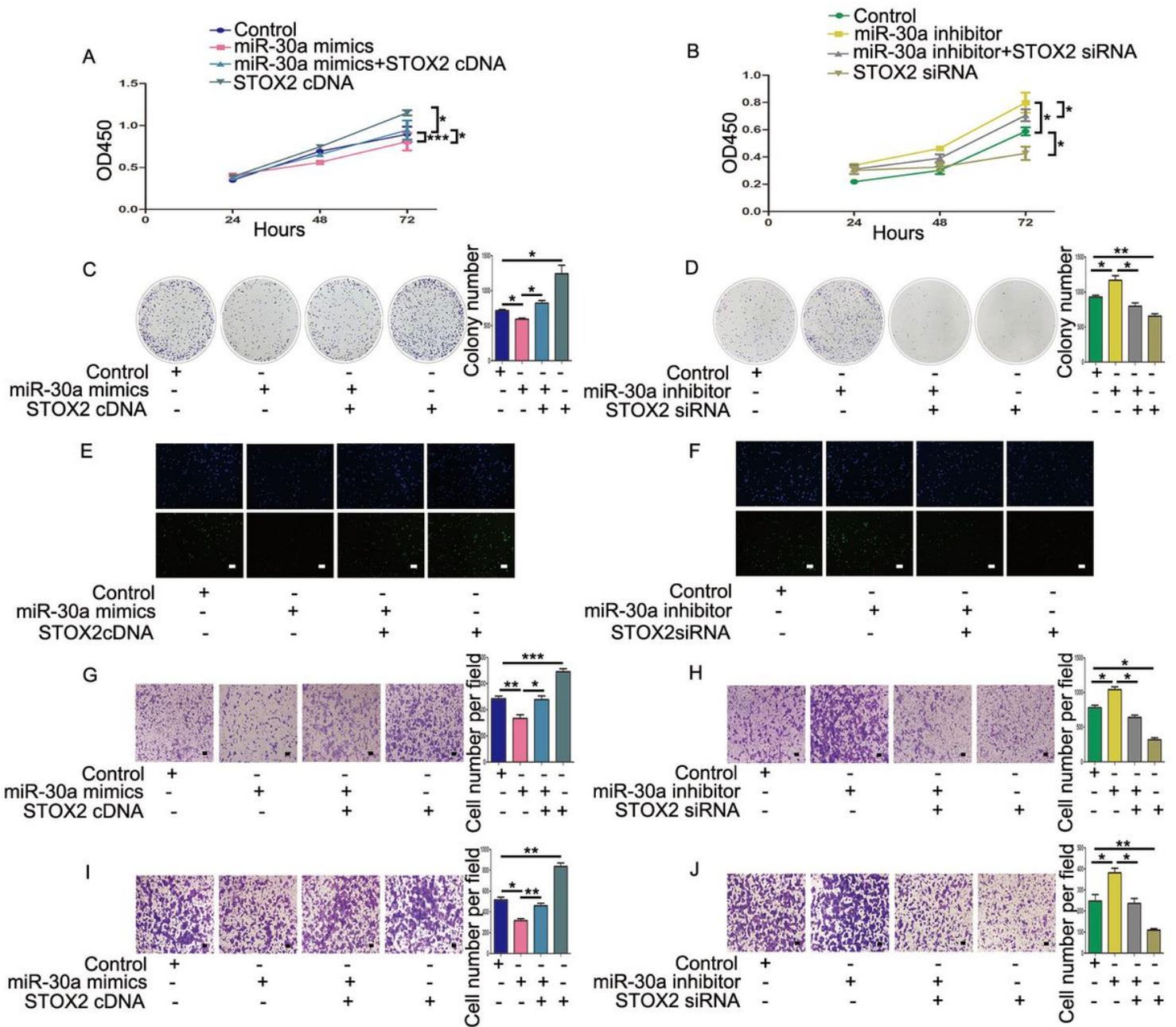


Figure 4

STOX2 mitigates miR-30a stimulation of proliferation and metastasis in JAR cells. (A and B) CCK-8 assay was showed that the proliferation of JAR cells by co-transfected miR-30a mimics and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA. (C and D) Colony formation experiment was tested the growth of JAR cells. (E and F) the vitalities ability of JAR cells was analyzed by EdU assay. (G and H) Cell migration was detected after co-transfected and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA into JAR cells. (I and J) Cell invasion was showed after co-transfected and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA into JAR cells. Bar=100µm. *P<0.05 or **P<0.01, ***P<0.001.

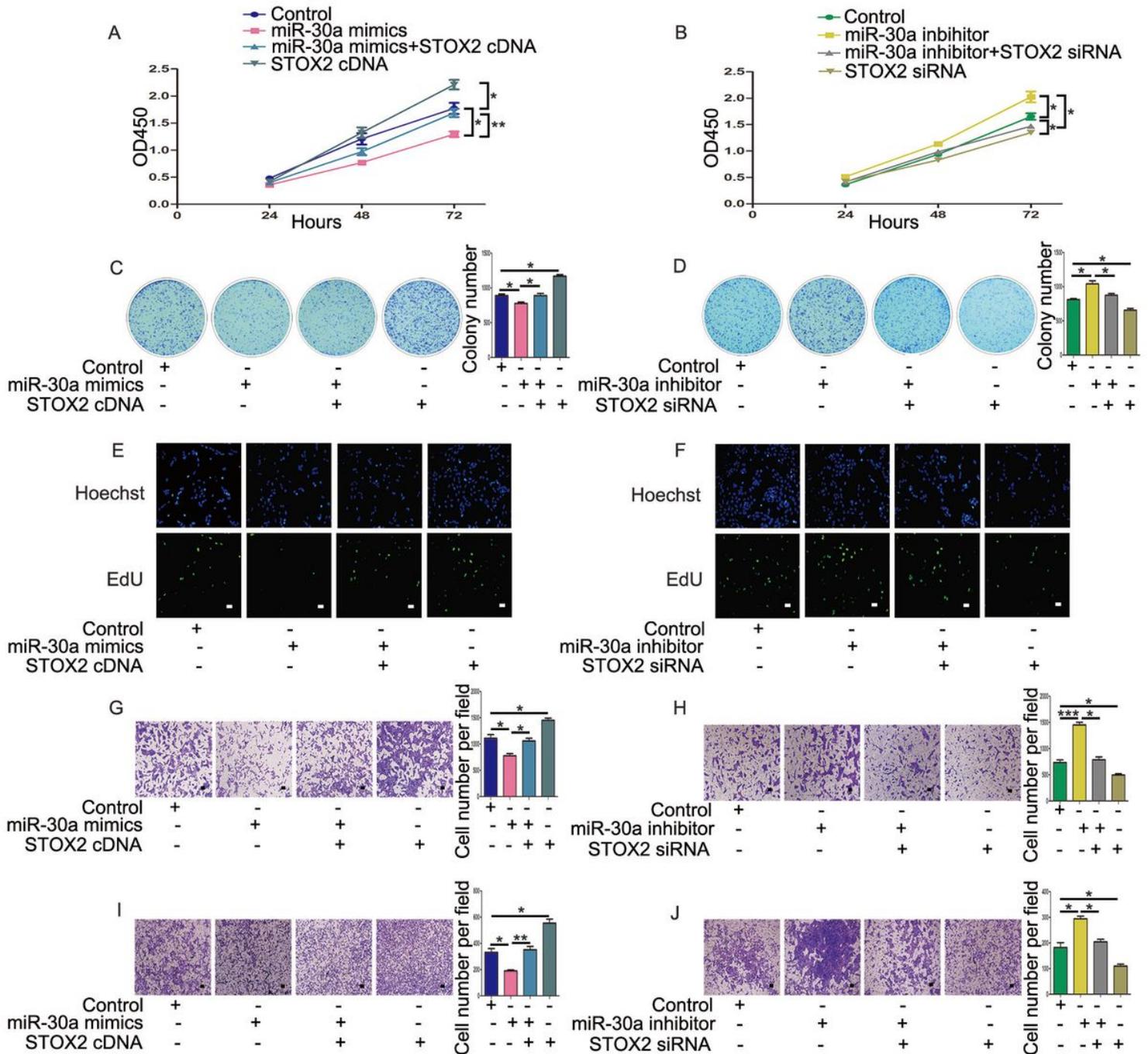


Figure 5

STOX2 mitigates miR-30a stimulation of proliferation and metastasis in HTR-8 cells. (A and B) CCK-8 assay was shown that the proliferation of HTR-8 cells by co-transfected miR-30a mimics and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA. (C and D) Colony formation experiment was tested the growth of HTR-8 cells. (E and F) the vitality ability of HTR-8 cells was analyzed by EdU assay. (G and H) Cell migration was found after co-transfected and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA into HTR-8 cells. (I and J) Cell invasion was shown after co-transfected and STOX2 cDNA or miR-30a inhibitor and STOX2 siRNA into HTR-8 cells. Bar=100µm. *P < 0.05 or **P < 0.01, ***P < 0.001.

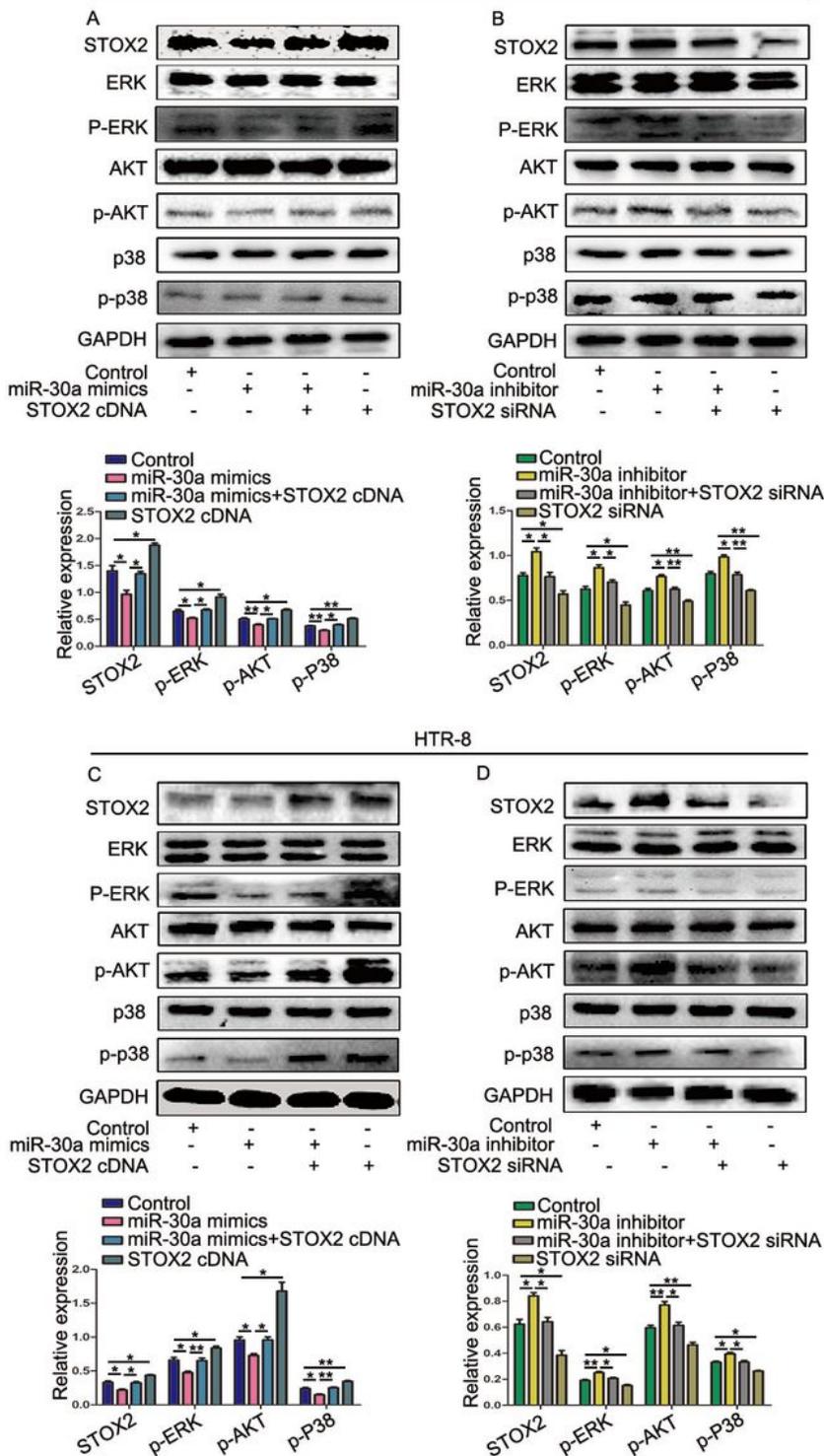


Figure 6

MiR-30a induces STOX2 expression and through ERK 1/2, AKT, and p38 Signaling Pathway in trophoblastic cells. (A and B) The protein expression of ERK, AKT, P38 signaling pathways-related proteins by co-transfected miR-30a mimics and STOX2 cDNA, and comparison of the expression of p-ERK1/2, p-AKT and p-P38 in each group. (C and D) The protein expression of ERK, AKT, P38 signaling

pathways-related proteins by co-transfected miR-30a inhibitor and STOX2 siRNA, and comparison of the expression of p-ERK1/2, p-AKT and p-P38 in each group. *P < 0.05 or **P < 0.01, ***P < 0.001.

Supplementary Files

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