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ATG4 promotes cell proliferation, migration and invasion in HCC and predicts a poor prognosis

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Abstract. – OBJECTIVE: ATG14, as an autophagy-related protein, has been shown to be implicated in the progression of tumors by modulating cell autophagy. We aimed at exploring ATG14 level in hepatocellular carcinoma (HCC) and its possible molecular mechanism.

PATIENTS AND METHODS: ATG14 levels in HCC tissues and cell lines were examined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), and the relationship between ATG14 expression and clinical parameters was analyzed through clinical information analysis. The impacts of ATG14 on the proliferation and invasiveness of HCC cells were evaluated by performing Cell Counting Kit-8 (CCK-8) and transwell tests, respectively. We further explored the potential mechanism of ATG14 action using bioinformatics analysis and *in vitro* cell experiments.

RESULTS: Our data showed that ATG14 levels were abnormally enhanced in HCC tissues and cell lines, which predicted a poor prognosis of HCC patients. Downregulation of ATG14 markedly blunted the proliferation and migratory capacities of HCC cells. Bioinformatics analysis suggested that XIST can regulate ATG14 by binding multiple miRNAs (miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p). In addition, XIST promoted cell autophagy by elevating ATG14 expression, thereby providing possible mechanisms by which ATG14 and XIST could modulate the development of HCC.

CONCLUSIONS: In summary, our data preliminary verified ATG14 levels were abnormally enhanced in HCC tissues and cell lines, which predicted a poor prognosis of HCC patients.

Key Words:
HCC, ATG14, XIST, Autophagy.

Introduction

Hepatocellular carcinoma (HCC), as the most common primary malignant tumor of the digestive system, has become the second leading cause of cancer death worldwide, with about one million new cases each year[1]. Hepatitis C virus (HCV) infection, long-term heavy drinking, non-alcoholic steatohepatitis and consumption of food contaminated with aflatoxin are all risk factors for this cancer[2]. The characteristics such as insidious onset, high malignancy, and rapid progression make it difficult for the early diagnosis of HCC. Although diversified and combined therapies for HCC have occurred, the long-term survival of HCC patients is still unsatisfying. Traditional tumor treatment methods such as surgical resection, interventional embolization, chemotherapy and radiotherapy are deficient for low cure rate and high recurrence and metastasis rate[3]. With the advent of the era of precision medicine, minimally invasive, efficient and individualized treatments gradually become the main direction of tumor therapy. Existing emerging molecular targeted drugs, such as more tyrosine kinase inhibitors, programmed cell death protein-1 antagonist; however, due to the limitations of the heterogeneity and complexity of the pathogenesis of HCC, the existing emerging molecular targeted drugs, such as more tyrosine kinase inhibitors, programmed cell death protein-1 antagonist, cannot achieve satisfactory results[4]. Therefore, it has become a hotspot to reveal the deep molecular signaling pathways related to HCC and to find new therapeutic targets.

In the known human genome, more than 60% of the genes can be transcribed into RNA, however, only less than 2% can be encoded into proteins. The rest of the gene transcripts that are not involved in protein coding due to lack of open reading frame are called non-coding RNAs[5]. Among which, long non-coding RNA is a class of non-coding RNA molecules, which are widely located in cell nucleus and cytoplasm. The mechanism of long non-coding RNA action is very complex and has not been fully understood[6].

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According to current studies, lncRNA can be engaged in a variety of cell biological processes including cell cycle, genomic localization, subcellular localization, epigenetic regulation, and gene modulation at transcriptional or post-transcriptional level[7].

Autophagy is an important process regulating the balance of active substances in cells and the homeostasis of the intracellular environment. It serves as a "cleaner" that digesters the senescent wastes through lysosomes and maintains the normal metabolism renewal in cells[8]. Once autophagy becomes abnormal, this homeostasis is disrupted. Autophagy can be engaged in the tumor process through a set of pathways[9]. Of note, tanshinone I can induce apoptosis and autophagy *via* inhibiting the PI3K/AKT/mTOR pathway[10], thus weakening the malignant biological characteristics of ovarian cancer. LncRNA NEAT1 knockdown weakens autophagy by targeting microRNA-34a[11], thus improving the 5-FU sensitivity to colorectal cancer cells. ATG14 has been proved to play an essential role in myeloma, ovarian cancer and osteosarcoma[12-14]; however, its function in HCC remains to be explored. In this study, we examined ATG14 expression level in HCC tissues and HCC cells for the first time, and discussed the possible molecular mechanism through which ATG14 could affect HCC progression.

Patients and Methods

Sample Collection

50 pairs of HCC tissue specimens were collected from our hospital and stored in -80°C refrigerator. The tissues obtained from the tumor 5 cm away from the tumor margin were confirmed as normal by pathology. The tumor pathological classification and staging standards of HCC are performed according to the Union for International Cancer Control (UICC) staging standards. Our investigation was approved for use by the Ethics Committee of the Second Affiliated Hospital of Nanjing Medical University. All subjects signed informed consent.

Cell Culture and Transfection

Human normal liver cell LO2 and liver cancer cell lines Hep-3B, MHCC-97H, SK-Hep-1, and SMMC-7721 were provided by American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin in a humid atmosphere at 37°C and 5% CO_2. For transient transfection, Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) was mixed with sh-ATG14 or microRNAs mimics when cell density reached to more than 70%.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from tissue specimens or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using PrimeScript ™ RT kit (TaKaRa, Otsu, Shiga, Japan). qPCR was carried out with the SYBR Green kit according to the product instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as internal references. The primer sequences are shown: ATG14 Forward: 5'-GCGCCAAATGCGTTCAGAG-3', Reverse: 5'-AGTCGGCTTAACCTTTCCTTCT-3'; miR-195-5p Forward: 5'-GGGGTAGCAGCACAGAAAT-3', Reverse: 5'-TCCAGTGCGTGTCGTGGA-3'; miR-497-5p Forward: 5'-CCTTCAGCAGCACACTGTGG-3', Reverse: 5'-CAGTGCAGGGTCCGAGGTAT-3'; miR-424-5p Forward: 5'-AACATTACACGCCAGACTTGCC-3', Reverse: 5'-GGTTTGACATTACTCAAGAGTTG-3'; miR-16-5p Forward: 5'-CTTAAGAACCCTCCTTACTC-3', Reverse: 5'-AAGCTACCCTAGGGGAAGGA-3'; GAPDH, forward: 5'-AGGTCGGTGTGAACGGATTTG-3', reverse: 5'-TGTAGACCATGTAGTTGAGGTCA-3'. U6 Forward: 5'-GCTGAGGTGACGGTCTCAAA-3', Reverse: 5'-GCCTCCCAGTTTCATGGACA-3'.

Cell Counting Kit-8 (CCK-8) Test

The cells were plated in 96-well plates ($4*10^3$ cells/well) in 100 uL culture medium. CCK-8 assay (Dojindo Molecular Technologies, Kumamoto, Japan) was performed according to the manufacturer's protocol.

Transwell Assay

Cell migration or invasion was tested using a 24-well plate cell (Corning Incorporated, Corning, NY, USA) pre-coated or not coated with matrix gel according to the manufacturer's instructions.

Luciferase Assay

ATG14-WT or ATG14-MUT (or XIST-WT or XIST-MUT) was constructed into pGL3-alkaline Luciferase vector (Promega Corporation, Madison, WI, USA). The Luciferase activity was measured using a Dual-Luciferase reporter kit (Promega, Madison, WI, USA).

Western Blot

Protein extraction was performed using a radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich, St. Louis, MO, USA). The protein samples were separated through sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland). Primary antibodies were incubated on the membrane to determine protein levels in the cells. After incubation of the corresponding secondary antibody, Image J software (NIH, Bethesda, MD, USA) was used for protein quantification.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism (Version X; La Jolla, CA, USA) were used for data analysis and mapping. Measurement data are presented as $x \pm$ SD (standard deviation). Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). The difference was statistically significant at $p<0.05$.

Results

HCC Tissues Contained Increased Expression of ATG14

We first detected ATG14 expression in HCC and adjacent control tissues by qPCR. Figure 1A shows a significant higher expression of ATG14 in HCC tissue samples. We, then, divided the subjects into TNM I + II group and TNM III + IV group according to TNM staging. It was found that ATG14 levels in tissues of the latter group were higher than those in the former (Figure 1B). Consistently, qPCR revealed that ATG14 was also abnormally overexpressed in HCC cell lines as compared to normal hepatic cells LO2 (Figure 1C). By analyzing the relationship between the survival of HCC patients and ATG14 expression, we found that high expression of ATG predicts a poor prognosis of liver cancer patients (Figure 1D). These results indicate that ATG14 may serve as a vital regulator in the progression of HCC.

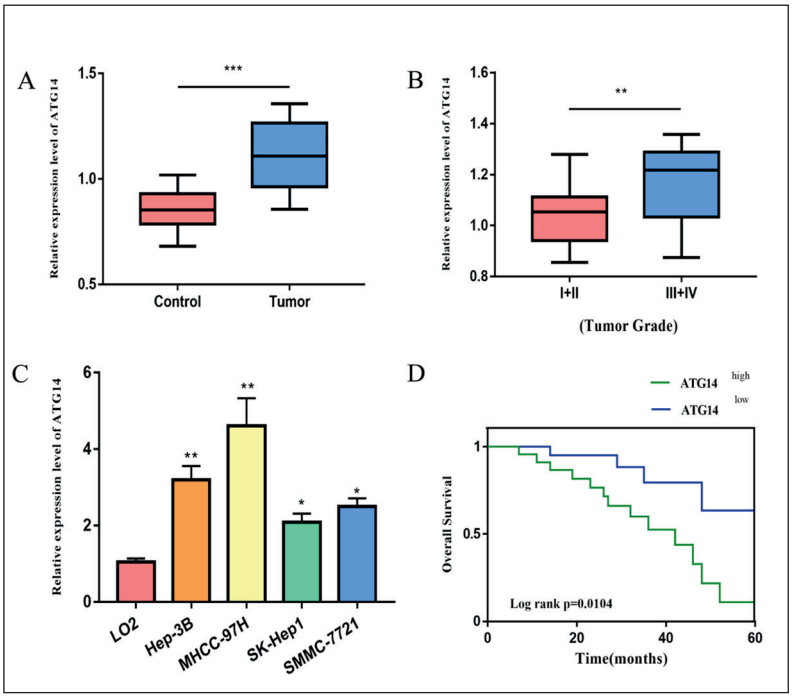

Figure 1. ATG14 is highly expressed in hepatocellular carcinoma tissues and cells. **A,** The relative expression level of ATG14 in hepatocellular carcinoma and normal control tissues was detected by qRT-PCR. **B,** The relative expression level of ATG14 in HCC tissues in different stages was detected by qRT-PCR. **C,** ATG14 expression was detected in hepatocellular carcinoma cell lines by qRT-PCR. **D,** The relationship between the expression level of ATG14 and the overall survival rate of patients was analyzed. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Down-Regulation of ATG14 Inhibits Proliferation Rate and Invasiveness of HCC Cells

To verify the biological function of ATG14, we resorted to the use of HCC cell lines MHCC-97H and Hep-3B with high ATG14 expression for *in vitro* researches. HCC cells were transfected with ATG14 siRNA (Figure 2A). Then, CCK8 and transwell assays revealed that downregulation of ATG14 markedly suppressed the proliferation and migration or invasiveness of MHCC-97H and Hep-3B cells, respectively (Figure 2B, 2C-2D). Taken together, these observations suggest that ATG14 can serve as an oncogene to promote the growth and metastasis of liver cancer cells.

XIST Regulates ATG14 Expression by Adsorbing Multiple miRNAs

We next sought to test the possible mechanism of ATG14 through searching for miRNAs that can bind to ATG14 in bioinformatics websites. As a result, a set of miRNAs including miR-15b-5p, miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p were found to have binding sites on ATG14 mRNA 3'UTR region (Figure 3A). Meanwhile, except miR-15b-5p has been reported to highly expressed in liver cancer, all of the other four miRNAs have been shown to have low expression in HCC and act as tumor-inhibiting genes[15-19]. We thus selected the four for subsequent research (Figure 3B). Figure 3C indicates that overexpression of these miRNAs effectively attenuate the Luciferase activity of ATG14-WT, while no significant difference was detectable in the ATG14-MUT group, indicating that these miRNAs can bind to ATG14 in HCC cells.

Subsequently, we predicted lncRNA XIST can simultaneously bind to the above four miRNAs through the bioinformatics analysis (Figure 3D-3E). Consistently, Figure 3C shows that overexpression of these miRNAs effectively reduces the Luciferase activity of XIST-WT, but there was no significant effect on the XIST-MUT group, indicating that these miRNAs can bind to XIST in HCC (Figure 3F). qPCR analysis indicated that upregulation of the four miRNAs markedly reduced ATG14 expression (Figure 3G). Furthermore, in HCC cells, inhibition of XIST was confirmed to significantly enhance the expression of

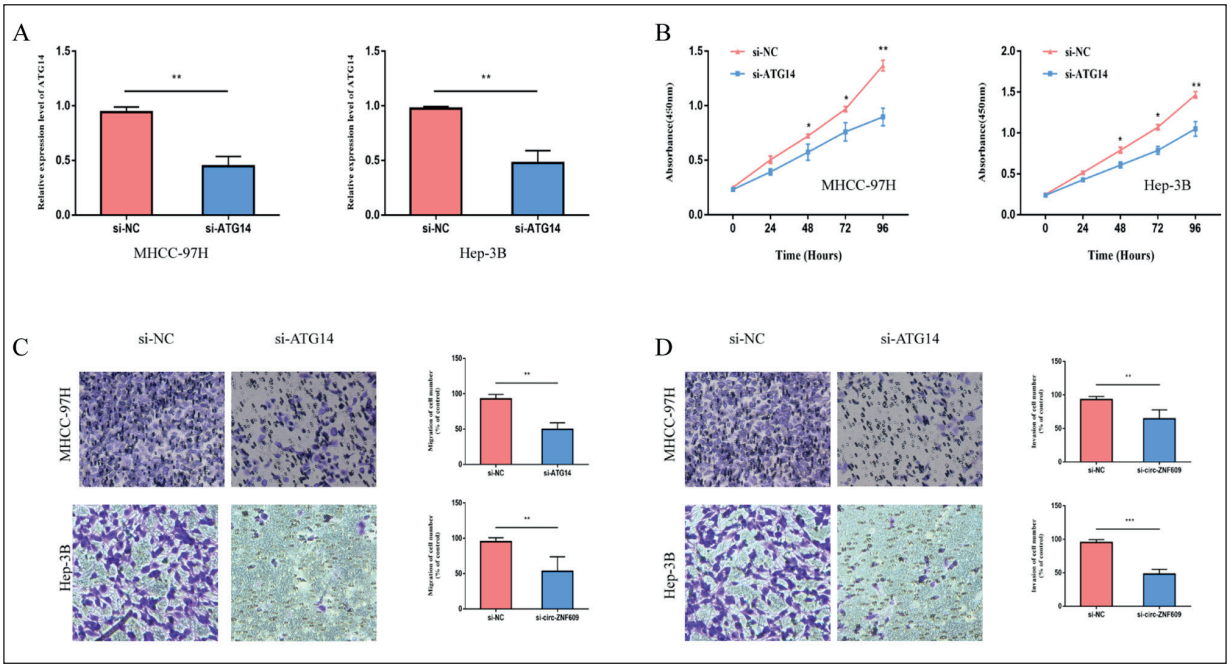

Figure 2. Downregulation of ATG14 can inhibit the proliferation, migration and invasion of hepatocellular carcinoma cells. **A,** qRT-PCR was used to detect the expression of ATG14 in MHCC-97H and Hep-3B cells transfected with si-NC and si-ATG14. **B,** The effect of ATG14 on the proliferation of MHCC-97H and Hep-3B cells was assayed by CCK8 experiment. **C,** The effect of ATG14 on the migration of MHCC-97H and Hep-3B cells was tested by transwell migration experiment (magnification: 20×). **D,** The effect of ATG14 on the invasion of MHCC-97H and Hep-3B cells was tested by transwell invasion experiment (magnification: 20x). **p*<0.05; ***p*<0.01; ****p*<0.001.

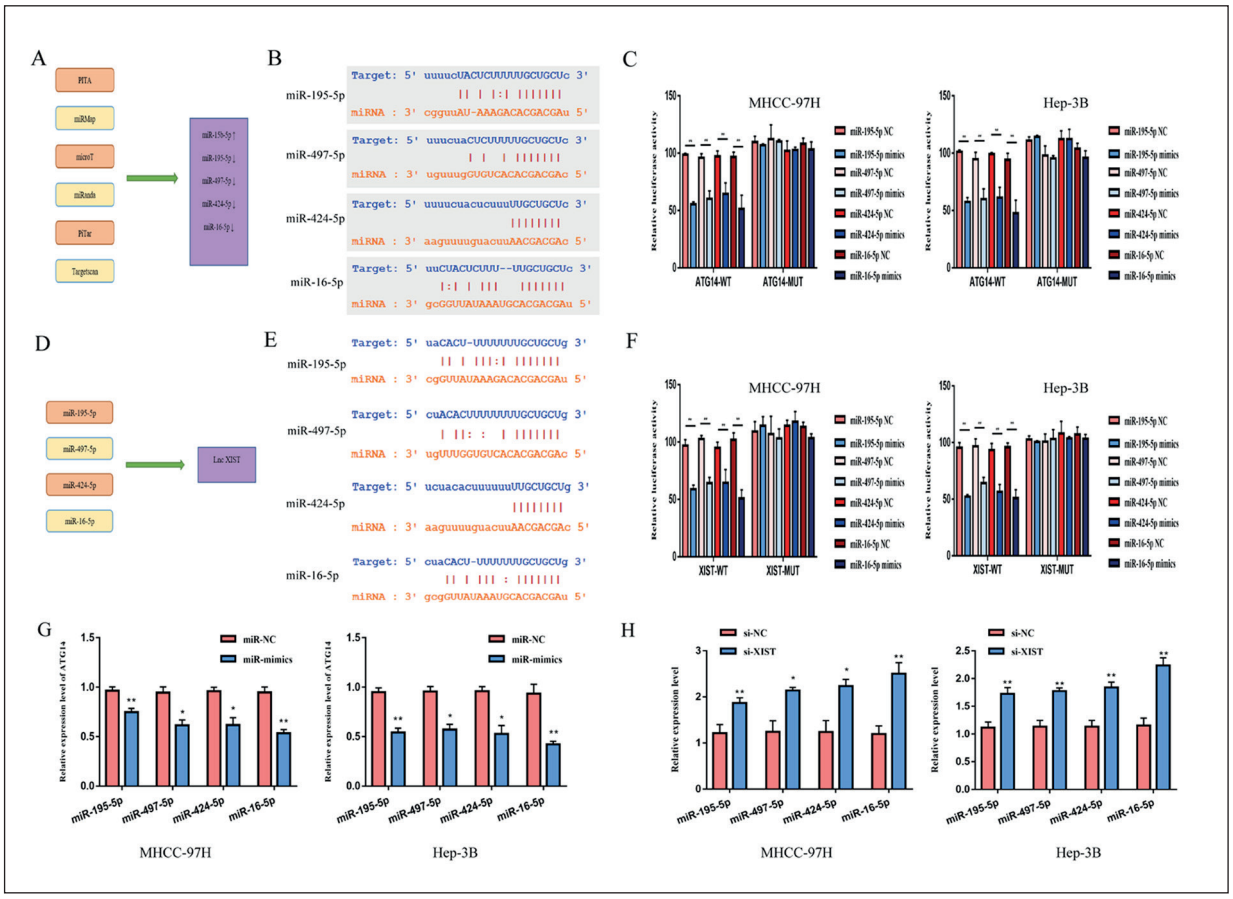

Figure 3. XIST can regulate ATG14 expression by adsorbing multiple miRNAs. **A,** Bioinformatics websites (PITA, miRMap, microT, miRanda, PiTar, and TragerScan) predict miRNAs that may bind to ATG14. **B,** Possible binding sites of ATG14 with miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p. **C,** After co-transfection of miRNAs (miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p) mimics in MHCC-97H and Hep-3B cells and containing ATG14-WT or ATG14-MUT, the Luciferase reporter activity is detected. **D,** Long-chain non-coding RNA is predicted to be capable of binding to miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p simultaneously. **E,** XIST possible binding sites with miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p. **F,** After co-transfection of miRNAs (miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p) mimics in MHCC-97H and Hep-3B cells and containing XIST-WT or XIST-MUT, the Luciferase reporter activity is detected. **G,** qRT-PCR detected ATG14 expression after transfection of miRNAs (miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p) mimics in MHCC-97H and Hep-3B cells. **H,** The expression of miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p were detected by qRT-PCR after transfection of MHCC-97H and Hep-3B with siNC and XIST siRNA. *p<0.05; **p<0.01.

these four miRNAs (Figure 3H). Taken together, our data indicate that XIST may elevate ATG14 level *via* binding multiple miRNAs, thereby affecting the progression of liver cancer.

XIST Promotes Cell Autophagy by Enhancing ATG14 Expression

To further confirm the regulation of XIST on ATG14, we knocked down or over-expressed XIST expression in MHCC-97H and Hep-3B cells and detected ATG14 expression by qPCR and Western blot. Figure 4A and 4B shows that knockdown of XIST significantly inhibits ATG14 expression at both mRNA and protein levels, while the opposite result was observed after XIST overexpression. Further, Western blot analysis revealed that downregulation of XIST inhibits the expression of cell autophagy-related protein LC3B while overexpression of XIST shows the converse effect. These results indicate that XIST may promote cell autophagy by promoting ATG14 expression (Figure 4C).

Discussion

Liver cancer has a high degree of malignancy and ranks second in mortality rate among

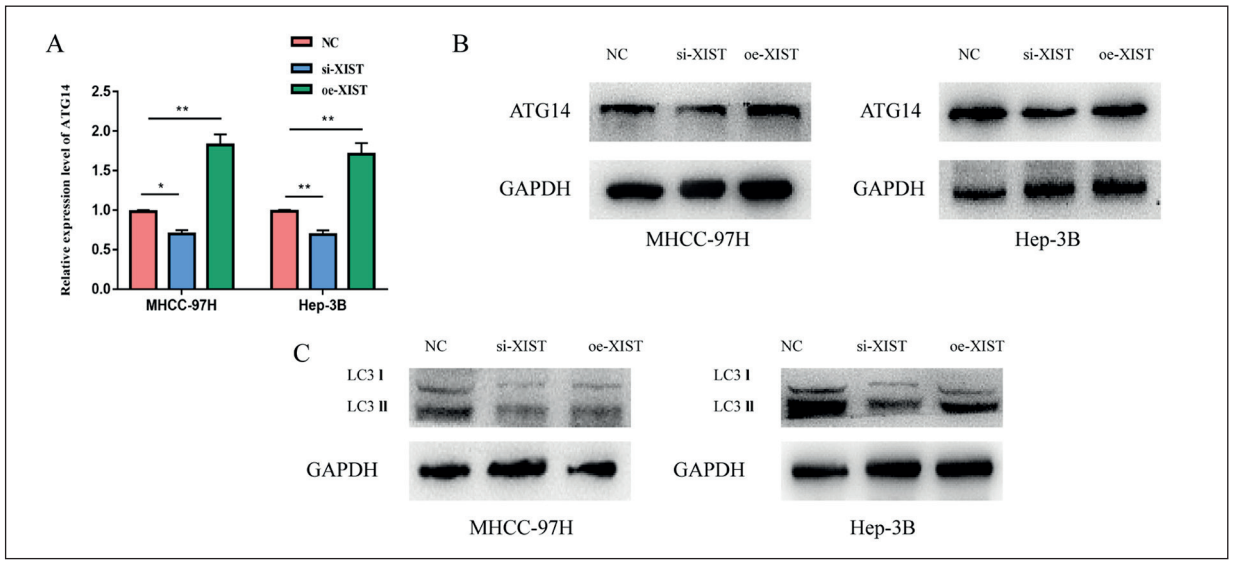

Figure 4. XIST improves cell autophagy by promoting ATG14 expression. **A,** The mRNA expression of ATG14 was detected by qRT-PCR after XIST was down-regulated or upregulated in MHCC-97H and Hep-3B cells. **B,** Western blot was used to detect the protein expression of ATG14 in MHCC-97H and Hep-3B cells after XIST was down-regulated or up-regulated. **C,** Western blot was used to observe cell autophagy in MHCC-97H and Hep-3B cells after XIST was down-regulated or up-regulated. **p*<0.05; ***p*<0.01.

all tumor diseases worldwide, just behind lung cancer. Chronic hepatitis B virus infection, aflatoxin, fatty liver induced by obesity, and hepatic cirrhosis caused by long-term drinking are the main factors leading to HCC[20]. The onset of this cancer is insidious, and its clinical diagnosis is a lack of scientific and effective indicators; thus, most HCC patients have been in advanced stage when they are diagnosed as HCC. The treatment of end-stage HCC patients is mainly interventional, immune, targeted and other therapeutic measures[21]. Therefore, it is precisely these characteristics of patients with HCC that endows the study on the mechanism of HCC an epoch-making significance.

LncRNA can achieve the regulation of target genes through apparent modification and transcriptional or post-transcriptional modulation. The current hot research content is the interaction between lncRNA and miRNA to achieve the targeted regulation of the target gene. LncRNA, as a competitive endogenous RNA (ceRNA), competes with miRNAs with same miRNA response element (MRE) to regulate gene expression and affect cell functions[22,23]. In the field of liver cancer research, several lncRNAs have been found to be involved in the genesis, development, and metastasis. Long-chain non-coding RNA RP5-833A20.1 inhibits tumorigenesis of liver cancer by targeting miR-18a-5p to regulate Akt/ERK pathway[24]; SNHG16 as miRNA let-7b-5p sponge regulates CDC25B in HCC and HMGA2 expression to promote G2/M and epithelial-mesenchymal transition[25]; lncRNA MIAT can be used as ceRNA, and in combination with miR-22-3p to up-regulate sirt1 to participate in the aging process of HCC cells[26]. It has been reported that lncXIST, abnormally overexpressed in HCC[27,28], could promote tumor cell growth and metastasis.

Autophagy, an important lysosomal degradation pathway in eukaryotic cell, serves as a dynamic recovery system and provides materials and energy for cells to synthesize new substances and maintain cell homeostasis[29]. The process of autophagy is precisely regulated by different proteins, and 40 autophagy-related genes have been identified so far. The activation of AMPK or the inhibition of mTOR leading to the activation of ULK1, which further phosphorylates Beclinl and activates VPS34. The complex of ULKl and Beclinl-VPS34 was then positioned on an open bilayer membrane structure. Autophagy precursors are extended by the At95-At912-At916L complex, with the continuous incorporation of LC3-II onto the autophagic membrane. After undergoing polyubiquitination, autophagy-targeted degradation substances are linked to LC3 II through a linker protein, and are then enveloped

by autophagic membranes to form closed vesicles called autophagosomes[9,30]. Finally, autophagosomes fuse with lysosomes to form autophagic lysosomes, which digest the contents and release the digested products into the cytoplasm[31].

In this study, ATG14 was found to be abnormally highly expressed in both HCC tissue samples and cells, which was also correlated with the tumor stage of HCC patients. By *in vitro* cell researches, we found that inhibiting ATG14 level can attenuate the proliferation ability, as well as cell invasion of HCC cells. Through bioinformatics analysis, we found that XIST can bind multiple miRNAs (miR-195-5p, miR-497-5p, miR-424-5p, and miR-16-5p) to regulate ATG14 expression. Further, we have confirmed that XIST may promote cell autophagy by promoting ATG14 level; however, the specific regulatory mechanism remains to be further studied *in vivo* experiments in the future.

Conclusions

In summary, our data preliminary verified ATG14 expression in HCC and its underlying mechanism of action, and provided a new perspective for the diagnosis and treatment of HCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Tsai MC, Yen YH, Chang KC, Hung CH, Chen CH, Lin MT, Hu TH. Elevated levels of serum urokinase plasminogen activator predict poor prognosis in hepatocellular carcinoma after resection. BMC Cancer 2019; 19: 1169.
2) Ai J, Ketabchi N, Verdi J, Gheibi N, Khadem HH, Kavianpour M. Mesenchymal stromal cells induce inhibitory effects on hepatocellular carcinoma through various signaling pathways. Cancer Cell Int 2019; 19: 329.
3) Liu Z, Yu Y, Huang Z, Kong Y, Hu X, Xiao W, Quan J, Fan X. CircRNA-5692 inhibits the progression of hepatocellular carcinoma by sponging miR-328-5p to enhance DAB2IP expression. Cell Death Dis 2019; 10: 900.
4) Dendy MS, Ludwig JM, Stein SM, Kim HS. Locoregional therapy, immunotherapy and the combination in hepatocellular carcinoma: future directions. Liver Cancer 2019; 8: 326-340.
5) Li Y, Han X, Feng H, Han J. Long noncoding RNA OIP5-AS1 in cancer. Clin Chim Acta 2019; 499: 75-80.
6) Chu F, Xue L, Miao H. Long noncoding RNA TP73-AS1 in human cancers. Clin Chim Acta 2020; 500: 104-108.
7) Yao C, Yu B. Role of long noncoding RNAs and circular RNAs in nerve regeneration. Front Mol Neurosci 2019; 12: 165.
8) Wen X, Klionsky DJ. At a glance: a history of autophagy and cancer. Semin Cancer Biol 2019. pii: S1044-579X(19)30167-1.
9) Ye X, Zhou XJ, Zhang H. Autophagy in immune-related renal disease. J Immunol Res 2019; 2019: 5071687.
10) Zhou J, Jiang YY, Chen H, Wu YC, Zhang L. Tanshinone I attenuates the malignant biological properties of ovarian cancer by inducing apoptosis and autophagy via the inactivation of PI3K/AKT/mTOR pathway. Cell Prolif 2020; 53: e12739.
11) Liu F, Ai FY, Zhang DC, Tian L, Yang ZY, Liu SJ. LncRNA NEAT1 knockdown attenuates autophagy to elevate 5-FU sensitivity in colorectal cancer via targeting miR-34a. Cancer Med 2020; 9: 1079-1091.
12) Lu D, Yang C, Zhang Z, Cong Y, Xiao M. Knockdown of linc00515 inhibits multiple myeloma autophagy and chemoresistance by upregulating miR-140-5p and downregulating ATG14. Cell Physiol Biochem 2018; 48: 2517-2527.
13) Zhao Z, Tao L, Shen C, Liu B, Yang Z, Tao H. Silencing of Barkor/ATG14 sensitizes osteosarcoma cells to cisplatin-induced apoptosis. Int J Mol Med 2014; 33: 271-276.
14) He J, Yu JJ, Xu Q, Wang L, Zheng JZ, Liu LZ, Jiang BH. Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. Autophagy 2015; 11: 373-384.
15) Zhang Y, Zhu Z, Huang S, Zhao Q, Huang C, Tang Y, Sun C, Zhang Z, Wang L, Chen H, Chen M, Ju W, He X. LncRNA XIST regulates proliferation and migration of hepatocellular carcinoma cells by acting as miR-497-5p molecular sponge and targeting PDCD4. Cancer Cell Int 2019; 19: 198.
16) Xu H, Hu YW, Zhao JY, Hu XM, Li SF, Wang YC, Gao JJ, Sha YH, Kang CM, Lin L, Huang C, Zhao JJ, Zheng L, Wang Q. MicroRNA-195-5p acts as an anti-oncogene by targeting PHF19 in hepatocellular carcinoma. Oncol Rep 2015; 34: 175-182.
17) Liu Z, Wang Y, Wang L, Yao B, Sun L, Liu R, Chen T, Niu Y, Tu K, Liu Q. Long non-coding RNA AGAP2-AS1, functioning as a competitive endogenous RNA, upregulates ANXA11 expression by sponging miR-16-5p and promotes proliferation and metastasis in hepatocellular carcinoma. J Exp Clin Cancer Res 2019; 38: 194.

18) Zhang Y, Li T, Guo P, Kang J, Wei Q, Jia X, Zhao W, Huai W, Qiu Y, Sun L, Han L. MiR-424-5p reversed epithelial-mesenchymal transition of anchorage-independent HCC cells by directly targeting ICAT and suppressed HCC progression. Sci Rep 2014; 4: 6248.

19) Pan WY, Zeng JH, Wen DY, Wang JY, Wang PP, Chen G, Feng ZB. Oncogenic value of microRNA-15b-5p in hepatocellular carcinoma and a bioinformatics investigation. Oncol Lett 2019; 17: 1695-1713.

20) Kapiris I, Nastos K, Karakatsanis A, Theodosopoulos T, Karandrea D, Kondi PA, Contis J. Survivin expression in hepatocellular carcinoma. Correlation with clinicopathological characteristics and overall survival. J BUON 2019; 24: 1934-1942.

21) Liu S, Qiu J, He G, Liang Y, Wang L, Liu C, Pan H. LncRNA MALAT1 acts as a miR-125a-3p sponge to regulate FOXM1 expression and promote hepatocellular carcinoma progression. J Cancer 2019; 10: 6649-6659.

22) Jin SJ, Jin MZ, Xia BR, Jin WL. Long Non-coding RNA DANCR as an emerging therapeutic target in human cancers. Front Oncol 2019; 9: 1225.

23) Greco S, Gaetano C, Martelli F. Long noncoding competing endogenous RNA networks in age-associated cardiovascular diseases. Int J Mol Sci 2019; 20: 3079.

24) Chen Z, Ma Y, Pan Y, Zuo S, Zhu H, Yu C, Zhu C, Sun C. Long noncoding RNA RP5-833A20.1 suppresses tumorigenesis in hepatocellular carcinoma through Akt/ERK pathway by targeting miR-18a-5p. Onco Targets Ther 2019; 12: 10717-10726.

25) Li S, Peng F, Ning Y, Jiang P, Peng J, Ding X, Zhang J, Jiang T, Xiang S. SNHG16 as the miRNA let-7b-5p sponge facilitates the G2/M and epithelial-mesenchymal transition by regulating CDC25B and HMGA2 expression in hepatocellular carcinoma. J Cell Biochem 2020; 121: 2543-2558.

26) Zhao L, Hu K, Cao J, Wang P, Li J, Zeng K, He X, Tu PF, Tong T, Han L. LncRNA MIAT functions as a ceRNA to upregulate sirt1 by sponging miR-22-3p in HCC cellular senescence. Aging (Albany NY) 2019; 11: 7098-7122.

27) Zhang Y, Zhu Z, Huang S, Zhao Q, Huang C, Tang Y, Sun C, Zhang Z, Wang L, Chen H, Chen M, Ju W, He X. LncRNA XIST regulates proliferation and migration of hepatocellular carcinoma cells by acting as miR-497-5p molecular sponge and targeting PDCD4. Cancer Cell Int 2019; 19: 198.

28) Kong Q, Zhang S, Liang C, Zhang Y, Kong Q, Chen S, Qin J, Jin Y. LncRNA XIST functions as a molecular sponge of miR-194-5p to regulate MAPK1 expression in hepatocellular carcinoma cell. J Cell Biochem 2018; 119: 4458-4468.

29) Dong S, Xiao Y, Ma X, He W, Kang J, Peng Z, Wang L, Li Z. MiR-193b increases the chemosensitivity of osteosarcoma cells by promoting FEN1-mediated autophagy. Onco Targets Ther 2019; 12: 10089-10098.

30) Mulcahy LJ, Thorburn A. Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients. Cell Death Differ 2020; 27: 843-857.

31) Furukawa K, Innokentev A, Kanki T. Regulatory mechanisms of mitochondrial autophagy: lessons from yeast. Front Plant Sci 2019; 10: 1479.