Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Inhibition of RNaseH2A Induces Fas-Regulated Programmed Cell Death in Hepatoma Cells

Xiaowei Chang, Yu Cai*, Pu Yan, Wujun Li

Department of General Surgery, The First Affiliated Hospital of Xi'an Medical University, Xi'an 710000, China

***Corresponding author:** Yu Cai, jxzlkzk@126.com

Abstract: *Objective:* To screen clinically significant potential drug targets in liver cancer and to study the function and potential molecular mechanisms of this target protein in the development of liver cancer. *Methods:* By using the clinical database GEPIA to find genes that are differentially expressed in liver cancer compared to normal tissues, we further screened the genes that are highly expressed in hepatocellular carcinoma and have clinical prognostic relevance. Heat maps were used to sort these genes according to their clinical prognostic relevance, so as to screen for the target gene of interest. The characteristics of target gene expression and clinical prognosis in hepatocellular carcinoma were studied. The target gene was knocked down through siRNA, and cell proliferation experiments and apoptosis experiments were used to verify the importance of the target gene in the occurrence and development of liver cancer. Finally, we elucidated the potential molecular mechanism of the target gene's function in liver cancer based on the mutual regulatory relationship between the target gene and key apoptosis genes. *Results:* 1482 genes were significantly underexpressed in liver cancer, and 725 genes were significantly overexpressed in liver cancer, of which RNaseH2A was significantly overexpressed in liver cancer and had a significant clinical prognosis. Knockdown of *RNaseH2A* inhibited the proliferation of hepatocellular carcinoma cells and induced apoptosis. Knockdown of *RNaseH2A* induced the high expression of *Fas*, a key gene for apoptosis, and liver cancer usually features low expression of *Fas*. After hepatocellular carcinoma cells that were knocked down of *RNaseH2A* continued were subject to *Fas* knockdown, hepatocellular carcinoma cell proliferation and apoptosis returned to normal levels. *Conclusion:* The high expression of *RNaseH2A* regulates the low expression of *Fas*, a key gene for apoptosis, thereby inhibiting apoptosis, promoting cell proliferation, and participating in the development of liver cancer.

Keywords: Liver cancer; RNaseH2A; Fas; Apoptosis

Online publication: May 30, 2022

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults with high malignancy and poor prognosis [1]. Treatment options and prognosis for HCC depend on many factors, such as tumor size and tumor malignancy. Surgical treatment does not produce obvious therapeutic effect on liver cancer, because only 10–20% of patients are amenable to treatment by surgical resection [2]. Chemotherapy is one of the currently available options for treating HCC but its therapeutic effect is limited. Therefore, the current focus of HCC research is to identify alternative therapeutic drugs with favorable pharmacological effects and low normal cytotoxicity [3].

RNaseH2A is also known as AGS4, JUNB, RNHL, RNIAA and RNaseHI. *RNaseH2A* gene (1148 bp) is located on chromosome 19 [4,5] and is a component of ribonuclease H2 (RNaseH2), mainly responsible for its endoribonuclease activity [6-8]. RNaseH was discovered and isolated from calf thymus [9,10], and is

widely distributed in mammalian cells, yeast, prokaryotes and viruses. It catalyzes the nuclear degradation of RNA in DNA-RNA hybrids, participates in the reverse transcription activity of multifunctional enzymes in retroviruses, and plays an important role in all stages of viral genome transcription [11,12]. In eubacteria, RNaseH is required for several processes, including removal of RNA primers from Okazaki fragments, transcription of primers required for DNA polymerase I to initiate DNA synthesis, and removal of R loops to provide for irregular DNA synthesis conditional start site [13]. In eukaryotes, RNaseH may play a similar role [14]. Recent studies have shown that RNaseH2A mutations cause autosomal recessive neurological dysfunction, Aicardi-Goutieres syndrome, mainly causing microcephaly, mental retardation, brain calcification, increased IF-α and leukocytes in cerebrospinal fluid, fever, thrombocytopenia, and hepatitis [15-17]. RNaseH2A is considered a possible cancer target. Consistently, logistic regression analysis showed that, among other genes, the expression level of RNaseH2A was positively correlated with aggressive prostate cancer [18]. However, the role of RNaseH2A in liver cancer has not been reported. Therefore, in this study, we aimed to evaluate the role of RNaseH2A in liver cancer and explore its underlying mechanism.

2. Materials and methods

2.1. Cell culture

Human hepatoma HepG2 cells were purchased from the ATCC cell bank in the United States, and were cultured in RPMI-1640 medium containing 10% fetal bovine serum, supplemented with 100 μg/mL streptomycin and 100 IU/ml penicillin. Cell culture flasks were placed in a 37°C incubator with 5% CO_2 and 95% humidity.

2.2. Clinical data screening

In the clinical database GEPIA (Gene Expression Profiling Interactive Analysis) [19], the proteins that are highly expressed in liver cancer were identified (tumor/normal>2) , and the proteins closely related to the clinical prognosis of liver cancer were found in the GEPIA database. Differential expression sorting was performed to screen for the target protein.

2.3. Real-time fluorescent quantitative PCR experiment (RT-qPCR)

Primer3 (http://frodo.wi.mit.edu/primer3/) was used to design specific qPCR primers. cDNA was diluted with sterilized pure water to an appropriate concentration, usually 20× dilution, according to the following recipe: qPCR mix 5 μL , primer 1 μL, and cDNA 4 μL. The reagent mixture was then added to a 96-well PCR plate with the film attached. The plate was centrifuged at 2500 rpm for 1 min, and the sample was put into the qPCR instrument for experimentation. After the reaction was completed, the data was copied for analysis.

2.4. Transfection

Six-well plate was used for transfection of siRNA. Two parts of reagent mixture were prepared: 1 μL siRNA mixed with 250 μL Opti-MEM, and 1 μL Lipo2000 mixed with 250 μL Opti-MEM. The reagents mixture parts were left at room temperature for 5 min, and then combined, mixed well, and let stand for 15 minutes.

2.5. Cell proliferation experiment

The overgrown cells were digested, resuspended in 1 ml fresh medium, and then plated with 20 μl at a density that can be plated in a 96- well plate. Each experimental group needed 3 biological replicates, and the required number of experimental wells were plated. The cells were incubated at 37°C with 5% CO_2. siRNA was transfected after cell adherence, and absorbance measurement was conducted after 48 h. The MTS reaction solution was prepared, and the reaction solution was prepared according to the ratio of

MTS:culture solution = 1:20. 100 μl of MTS reaction solution was added to each well, and the culture was continued at 37°C Absorbance was measured every half an hour. The petri dish was shaken at low speed for 10 s on a shaker to fully dissolve the crystals. The absorbance of each well was measured at 490 nm. The daily absorbance values were converted into parameters representing daily cell growth, and cell proliferation curves were plotted.

2.6. Apoptosis experiment

Apoptosis kits (BDscience, New Jersey, USA) were used to detect the apoptosis levels of cells in each group. Cells transfected with siRNA were digested and centrifuged at 1000 rpm for 5 min, washed with phosphate-buffered saline, centrifuged twice, and resuspended in 100μl of 1× Binding buffer. 5 μl of propidium iodide (PI) and 5 μl of Anexin V were added. The cells were incubated at room temperature for 15 min in the dark, and then sent to the scientific research center of a hospital within 1 h for on-machine testing.

2.7. Western blot experiment

The cells were collected for protein quantification and sample preparation. Electrophoresis, electrotransfer device, electrophoresis solution and electrotransfer solution were prepared as per standard procedures. The samples were loaded into the gel, and electrophoresis was conducted at 100 V. The electrophoresis process was terminated as soon as the blue band ran out. The electrotransfer process was carried out at 100 V for 2 h, and the protein was transferred. The membrane was blocked in milk for 1 h, incubated with primary antibody overnight at 4°C, washed 4 times with 5 min each time, incubated with secondary antibody at room temperature for 1 h, washed 4 times with 5 min each time, and eventually developed in the dark room.

3. Result

3.1. Screening of clinically significant target genes in liver cancer

In order to find the target genes with significant clinical significance in liver cancer, we used the GEPIA database to find genes with significant differential expression in liver cancer and adjacent normal tissues, of which 725 were overexpressed and 1482 were underexpressed in liver cancer (as shown in **Figure 1A**). The 725 genes that were overexpressed in liver cancer were ranked according to their clinical prognosis. We looked for genes in liver cancer according to the principle of high gene expression and poor prognosis, combined with the differential expression folds between liver cancer and normal tissues (**Figure 1B**). We found that RNaseH2A has clinical significance.

3.2. Expression characteristics of RNaseH2A in liver cancer

We found a clinically significant target gene *RNaseH2A* in liver cancer. First, we found that the expression of *RNaseH2A* was indeed significantly higher in liver cancer than in normal tissues through the GEPIA database (as shown in **Figure 2A**). At the same time, we found that RNaseH2A has a significant prognosis in liver cancer. As shown in **Figure 2B**, the expression of RNaseH2A also increased with the increase of the malignancy degree of liver cancer (**Figure 2C**), which was significantly higher than that of normal tissues. These characteristics indicate that RNaseH2A has an important role in liver cancer.

3.3. Knockdown of *RNaseH2A* expression can significantly slow down the proliferation of hepatoma cells and induce apoptosis

In order to verify the important role of *RNaseH2A* in liver cancer, we used siRNA interference to reduce the expression of *RNaseH2A* in liver cancer cells (as shown in **Figure 3A**). Apoptosis experiments showed that knocking down the expression of *RNaseH2A* significantly induced apoptosis (**Figures 3C** and **3D**).

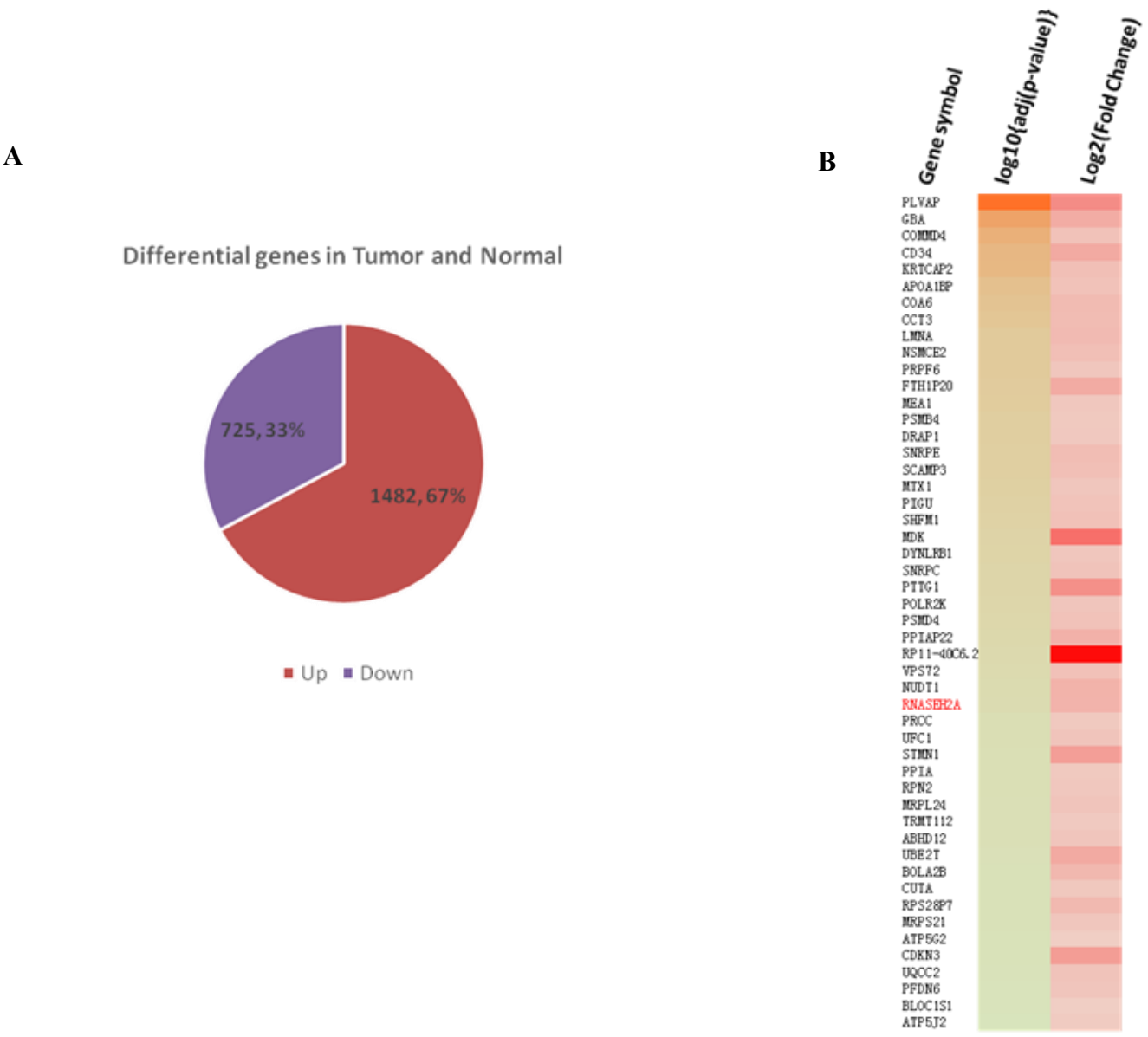

Figure 1. Screening of target genes with significant clinical significance in liver cancer; **A.** The number of differentially expressed genes in liver cancer compared with adjacent normal tissues through GEPIA database; **B.** Heat map analysis of the fold ranking of highly expressed genes in liver cancer

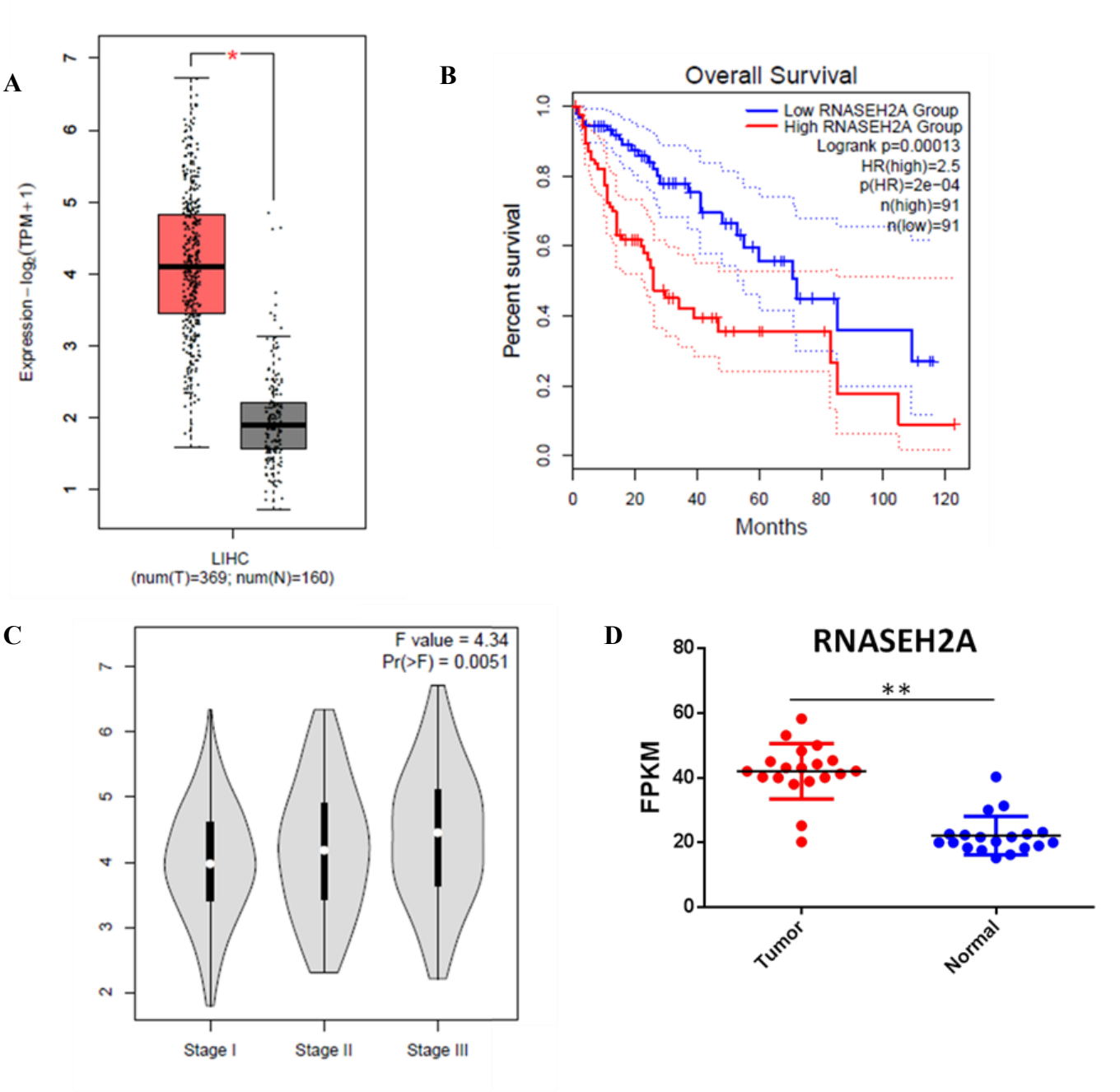

Figure 2. Expression characteristics of RNaseH2A in liver cancer; **A.** The GEPIA database shows the expression of RNaseH2A in liver cancer and adjacent normal tissues; **B.** The correlation between RNaseH2A and the clinical prognosis of liver cancer; **C.** The expression of *RNaseH2A* with the increase of the malignant degree of liver cancer; **D.** The expression of *RNaseH2A* in the sample

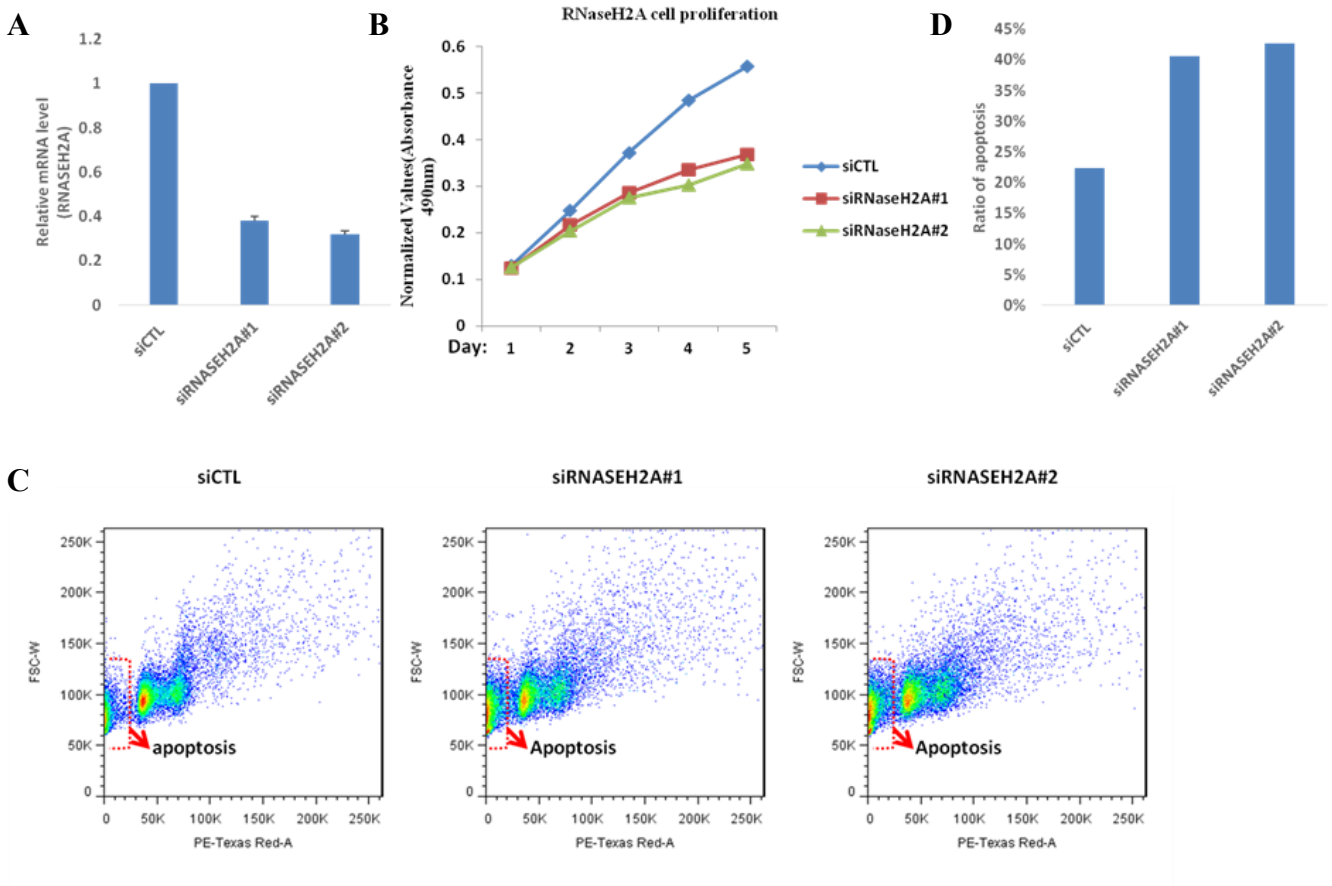

Figure 3. Knockdown of *RNaseH2A* expression can significantly slow down the proliferation of hepatoma cells and induce apoptosis; **A.** The knockdown efficiency of *RNaseH2A* expression by siRNA interference; **B.** The effect of knockdown of *RNaseH2A* expression on cell proliferation rate; **C** and **D.** The effect of knockdown of *RNaseH2A* expression on apoptosis

3.4. Knockdown of *RNaseH2A* can significantly induce the expression of *Fas*

Since the knockdown of RNaseH2A expression can significantly induce apoptosis, we tested the effect on the key gene *Fas* in apoptosis of liver cancer cells which were knocked down of *RNaseH2A* expression. We found that the knockdown of *RNaseH2A* expression significantly increased *Fas* RNA level and Fas protein level (**Figures 4A** and **4B),** showing a negative correlation between *RNaseH2A* and *Fas*. Consistent with this result, *RNaseH2A* was significantly overexpressed in HCC, while *Fas* was significantly underexpressed (**Figure 4C**), and this shows a significant negative correlation with the expression of *RNaseH2A* (**Figure 4D**).

3.5. *RNaseH2A* promotes cell proliferation and attenuates apoptosis by negatively regulating *Fas*

Because of the negative correlation between *RNaseH2A* and *Fas*, knockdown of *RNaseH2A* would induce high expression of *Fas*, thereby promoting apoptosis. Therefore, in order to verify that RNaseH2A directly plays an important role in liver cancer through Fas, we knocked down *RNaseH2A* expression in liver cancer cells. The knockdown of *Fas* expression (as shown in **Figure 5A**) caused the cell proliferation rate returned to the normal level (as shown in **Figure 5B**), and at the same time, abrogated the apoptosis caused by the knockdown of *RNaseH2A* (as shown in **Figure 5C**). These findings all point to the notion that *RNaseH2A* is a direct negative regulator of *Fas*, and it can promote the proliferation of liver cancer cells and slow down the apoptosis of cells to promote the development of liver cancer.

4. Discussion

Gene dysregulation is a hallmark of tumorigenesis and progression [20], and post-transcriptional regulation of mRNA is a key step. Ribonucleases catalyze the breakdown of RNA, thereby affecting mRNA turnover and gene expression; their dysfunction is associated with various types of tumors. For example, a failure to recruit poly(A)-specific ribonuclease (PARN), a polyribonuclease, has been observed in malignant gliomas [21]. In addition, primary osteosarcoma and its derived cell lines also have reduced expression in XRN1, a 5'-3' exonuclease that initiates mRNA degradation [22]. Furthermore, genes encoding RNase L truncations are positively associated with hereditary prostate cancer [23], while modest reductions in enzymatic activity are associated with a higher risk of prostate and colorectal cancer [24] and pancreatic cancer [25]. Inositol-requiring enzyme 1 (IRE1) is a transmembrane endoribonuclease found in the endoplasmic reticulum [26], which acts as a tumor suppressor and determines the fate of cancer cells [27]. Ribonucleases from miRNA pathways, including Drosha, Dicer and Ago2, have also been implicated in tumor biology. For example, Drosha expression was found to be elevated in esophageal cancer [28], and its inhibitory effect resulted in a reduction in cancer cell proliferation. In addition, elevated mRNA levels and genome copy number of Drosha were found in clinical cervical squamous cell carcinoma samples and derived cell lines [29]. Studies report that Dicer is significantly overexpressed in a variety of cancers, including salivary gland, lung, prostate and ovarian cancers as well as Burkitt lymphoma. Ago2 overexpression was also detected in these cancers [30].

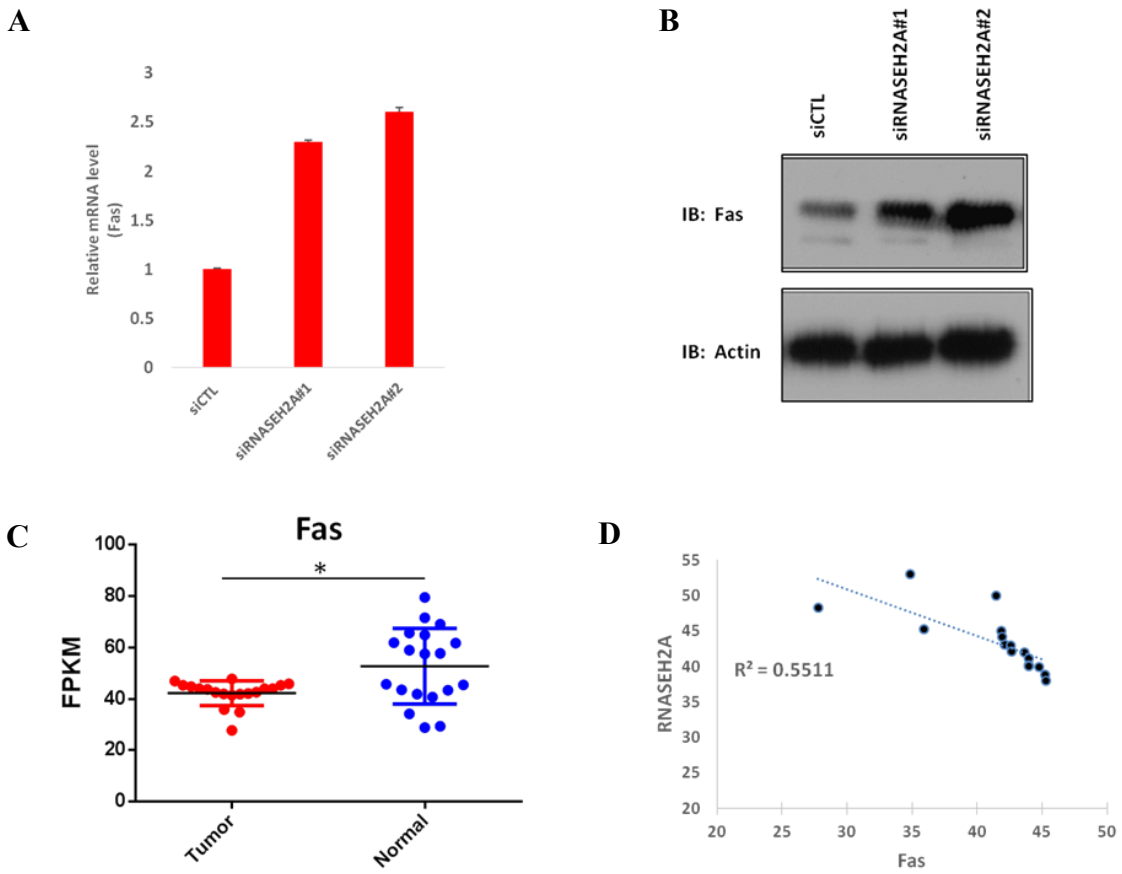

Figure 4. Knockdown of *RNaseH2A* can significantly induce the expression of *Fas*; **A.** Effect of knockdown of *RNaseH2A* expression on *Fas* RNA level; **B.** Effect of knockdown of *RNaseH2A* expression on FAS protein level; **C.** Expression of *Fas* in 19 groups of liver cancer clinical samples; **D.** *RNaseH2A* correlation with *Fas* expression in group 19 of the liver cancer clinical samples

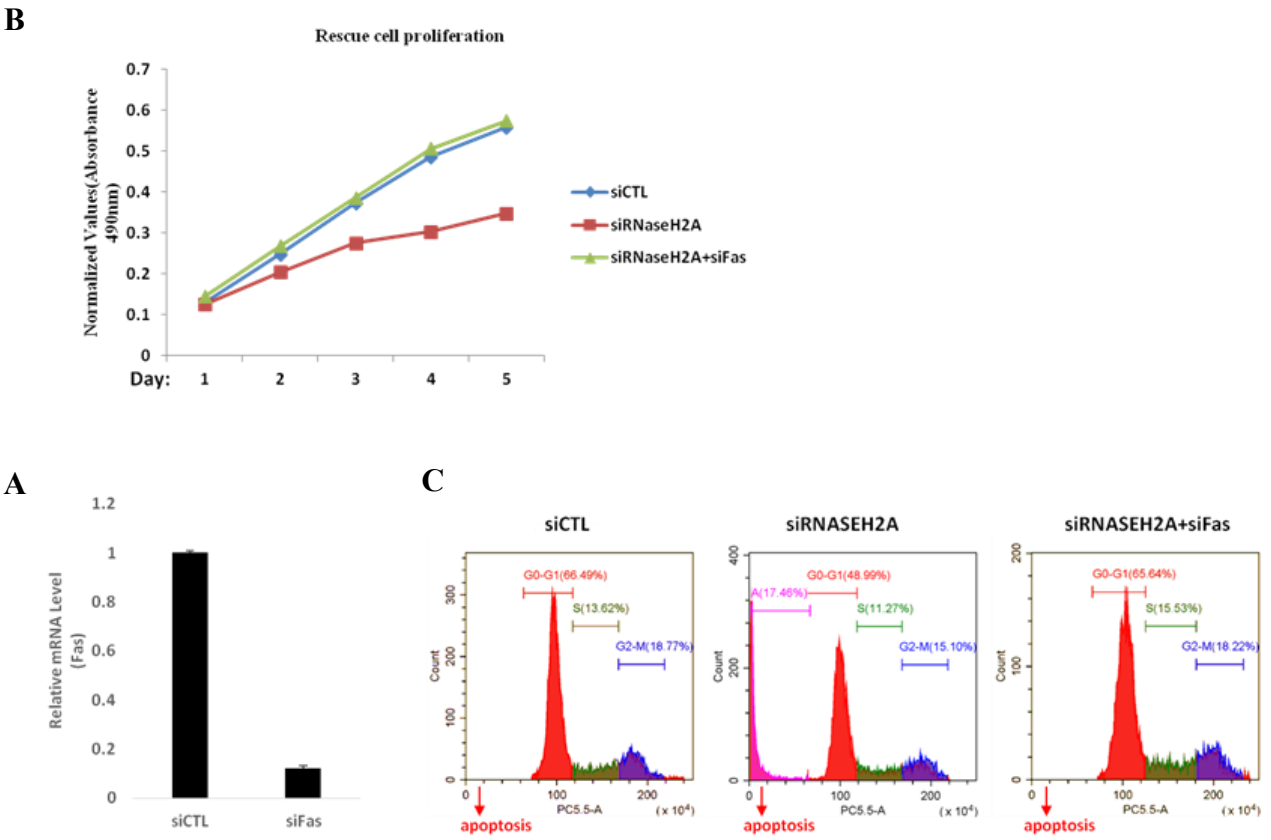

Figure 5. *RNaseH2A* promotes cell proliferation and attenuates apoptosis by negatively regulating *Fas*; **A.** The knockdown efficiency of siRNA interference to knock down *Fas* expression; **B.** The effect of simultaneous knockdown of *RNaseH2A* and *Fas* on the proliferation rate of liver cancer cells; **C.** The effect of simultaneous knockdown of *RNaseH2A* and *Fas* on the apoptosis of liver cancer cells

This article mainly explains the function and molecular mechanism of RNaseH2A in promoting the development of liver cancer. First, we screened *RNaseH2A* based on the fold difference of gene expression between liver cancer and clinically normal tissues in combination with the correlation with clinical prognosis, and further analyzed the database and we found from the clinical samples that *RNaseH2A* was significantly highly expressed in liver cancer, and had a significant clinical prognosis correlation. At the same time, with the increase of the malignancy degree of liver cancer, the expression of *RNaseH2A* increased, and then the expression of *RNaseH2A* was knocked down in liver cancer cells. It was found that it can inhibit cell proliferation and induce cell apoptosis, and cell apoptosis is mainly induced by knocking down the expression of *RNaseH2A*, which can induce the expression of *Fas*, a key gene of apoptosis. This suggests a negative correlation between *RNaseH2A* and *Fas*. *RNaseH2A* is highly expressed in liver cancer, while the expression of *Fas* is significantly lower in liver cancer. In order to further verify the direct regulatory relationship between *RNaseH2A* and *Fas* in liver cancer, we conducted a series of rescue assays and found that knockdown of *RNaseH2A* leads to increased *Fas* expression in liver cancer cells. With further knockdown of *Fas*, the cell proliferation rate and apoptosis returned to normal levels.

5. Conclusion

In conclusion, *RNaseH2A* promotes cell proliferation and slows down cell apoptosis, thereby promoting the progression of liver cancer mainly by negatively regulating the expression of the key apoptosis gene *Fas*.

Funding

General Special Scientific Research Project of Shaanxi Provincial Department of Education "Molecular Mechanism of MiR-122 Competing Endogenous lncRNA in the Development of Liver Cancer" (Project Number: 20JK0887)

Disclosure statement

The authors declare no conflict of interest.

References

[1] Balogh J, Victor D, Asham EH, et al, 2016, Hepatocellular Carcinoma: A Review. J Hepatocell Carcinoma, 3(1): 41-53.

[2] Attwa MH, El-Etreby SA, 2015, Guide for Diagnosis and Treatment of Hepatocellular Carcinoma. World J Hepatol, 7(12): 1632-1651.

[3] Kumar M, Panda D, 2014, Role of Supportive Care for Terminal Stage Hepatocellular Carcinoma. J Clin Exp Hepatol, 4(Suppl 3): S130-S139.

[4] Moelling K, Broecker F, 2015, The Reverse Transcriptase-RNase H: From Viruses to Antiviral Defense. Ann NY Acad Sci, 1341(4): 126-135.

[5] Natiq A, Elalaoui SC, Miesch S, et al., 2014, A New Case of De Novo 19p13.2p13.12 Deletion in a Girl with Overgrowth and Severe Developmental Delay. Mol Cytogenet, 7(1): 40.

[6] Flanagan JM, Funes JM, Henderson S, et al., 2009, Genomics Screen in Transformed Stem Cells Reveals RNASEH2A, PPAP2C, and ADARB1 as Putative Anticancer Drug Targets. Mol Cancer Ther, 8(1): 249-260.

[7] Feng S, Cao Z, 2016, Is the Role of Human RNase H2 Restricted to its Enzyme Activity? Prog Biophys Mol Biol, 121(1): 66-73.

[8] Reijns MA, Bubeck D, Gibson LC, et al., 2011, The Structure of the Human RNase H2 Complex Defines Key Interaction Interfaces Relevant to Enzyme Function and Human Disease. J Biol Chem, 286(12): 10530-10539.

[9] Hausen P, Stein H, 1970, Ribonuclease H: An Enzyme Degrading the RNA Moiety of DNA-RNA Hybrids. Eur J Biochem, 14(2): 278-283.

[10] Stein H, Hausen P, 1969, Enzyme from Calf Thymus Degrading the RNA Moiety of DNA-RNA Hybrids: Effect on DNA-Dependent RNA Polymerase. Science, 166(3903): 393-395.

[11] Coté ML, Roth MJ, 2008, Murine Leukemia Virus Reverse Transcriptase: Structural Comparison with HIV-1 Reverse Transcriptase. Virus Res, 134(1-2): 186-202.

[12] Mizuno M, Yasukawa K, Inouye K, 2010, Insight into the Mechanism of the Stabilization of Moloney Murine Leukaemia Virus Reverse Transcriptase by Eliminating RNase H Activity. Biosci Biotechnol Biochem, 74(2): 440-442.

[13] Schultz SJ, Champoux JJ, 2008, RNase H Activity: Structure, Specificity, and Function in Reverse Transcription. Virus Res, 134(1-2): 86-103.

[14] Rice GI, Forte GM, Szynkiewicz M, et al., 2013, Assessment of Interferon-Related Biomarkers in Aicardi-Goutières Syndrome Associated with Mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: A Case-Control Study. Lancet Neurol, 12(2): 1159-1169.

[15] Orcesi S, La Piana R, Fazzi E, 2009, Aicardi-Goutieres Syndrome. Br Med Bull, 89(1): 183-201.

[16] Crow YJ, 2013, Aicardi-Goutières Syndrome. Handb Clin Neurol, 113(1): 1629-1635.

[17] Crow YJ, Manel N, 2015, Aicardi-Goutières Syndrome and the Type I Interferonopathies. Nat Rev Immunol, 15(7): 429-440.

[18] Williams KA, Lee M, Hu Y, et al, 2014, A Systems Genetics Approach Identifies CXCL14, ITGAX, and LPCAT2 as Novel Aggressive Prostate Cancer Susceptibility Genes. PLoS Genet, 10(11): e1004809.

[19] Tang Z, Li C, Kang B, et al., 2017, GEPIA: A Web Server for Cancer and Normal Gene Expression Profiling and Interactive Analyses. Nucleic Acids Research, 45(1): 98-102.

[20] Yun K, Fidler AE, Eccles MR, et al., 1993, Insulin-Like Growth Factor II and WT1 Transcript Localization in Human Fetal Kidney and Wilms' Tumor. Cancer Res, 53(21): 5166-5171.

[21] Suswam E, Li Y, Zhang X, et al., 2008, Tristetraprolin Downregulates Interleukin-8 and Vascular Endothelial Growth Factor in Malignant Glioma Cells. Cancer Res, 68(3): 674-682.

[22] Zhang K, Dion N, Fuchs B, et al., 2002, The Human Homolog of Yeast SEP1 is a Novel Candidate Tumor Suppressor Gene in Osteogenic Sarcoma. Gene, 298(2): 121-127.

[23] Rökman A, Ikonen T, Seppälä EH, et al., 2002, Germline Alterations of the RNASEL Gene, a Candidate HPC1 Gene at 1q25, in Patients and Families with Prostate Cancer. Am J Hum Genet, 70(5): 1299-1304.

[24] Krüger S, Silber AS, Engel C, et al., 2005, German Hereditary Non-Polyposis Colorectal Cancer Consortium: Arg462Gln Sequence Variation in the Prostate-Cancer-Susceptibility Gene RNASEL and Age of Onset of Hereditary Non-Polyposis Colorectal Cancer: A Case-Control Study. Lancet Oncol, 6(8): 566-572.

[25] Bartsch DK, Fendrich V, Slater EP, et al., 2005, RNASEL Germline Variants are Associated with Pancreatic Cancer. Int J Cancer, 117(5): 718-722.

[26] Sidrauski C, Walter P, 1997, The Transmembrane Kinase Ire1p is a Site-Specific Endonuclease that Initiates mRNA Splicing in the Unfolded Protein Response. Cell, 90(6): 1031-1039.

[27] Davies MP, Barraclough DL, Stewart C, et al., 2008, Expression and Splicing of the Unfolded Protein Response Gene XBP-1 are Significantly Associated with Clinical Outcome of Endocrine-Treated Breast Cancer. Int J Cancer, 123(1): 85-88.

[28] Sugito N, Ishiguro H, Kuwabara Y, et al., 2006, RNAseN Regulates Cell Proliferation and Affects Survival in Esophageal Cancer Patients. Clin Cancer Res, 12(24): 7322-7328.

[29] Muralidhar B, Goldstein LD, Ng G, et al., 2007, Global Microrna Profiles in Cervical Squamous Cell Carcinoma Depend on Drosha Expression Levels. J Pathol, 212(4): 368-377.

[30] Kaul D, Sikand K, 2004, Defective RNA-Mediated c-myc Gene Silencing Pathway in Burkitt's Lymphoma. Biochem Biophys Res Commun, 313(3): 552-554.