MiRNA-485-5p inhibited the proliferation and promoted the apoptosis of hepatocellular carcinoma cells by targeting MUC1

Ming Li
Hunan University of Medicine
Dong Zhu
Hunan University of Medicine
Xiaozhen Peng
Hunan University of Medicine
Pinyue Liu
Hunan University of Medicine
Fen Yang
Hunan University of Medicine
Hai Luo
Hunan University of Medicine
Xuemei Zhang
Hunan University of Medicine
Fang Liu
Hunan University of Medicine
Le Wei (weile628@163.com)
Hunan University of Medicine

Research

Keywords: MiRNA-485-5p, MUC1, proliferation, apoptosis, hepatocellular carcinoma

Posted Date: April 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20589/v1

Abstract

Background The downregulation of miRNA-485-5-p was related to prognosis of various cancers, including hepatocellular carcinoma (HCC), while MUC1 was aberrantly expressed in many cancers and could be a biomarker for prognosis and diagnosis of cancers. The aim of the study was to investigate whether the miRNA-485-5p inhibited proliferation and promoted apoptosis by targeting MUC1 in HCC cells.

Methods The expression of miRNA-485-5p in patients with HCC was measured by qPCR, and the epxression of MUC1 was detected by qPCR, western blot and immunohistochemistry. Bioinformatics and dual luciferase assay verified the targeting relationship between miRNA-485-5p and MUC1 in HCC cells. Cell proliferation was detected by CCK-8 assay. Cell apoptosis was detected by flow cytometry.

Results The expression of miRNA-485-5p in HCC tissues is downregulated than that in adjacent tissue. However, the exprssion of MUC1 is opposite to the expression of miRNA-485-5p in the patients with HCC. Silencing MUC1 and overexpression miRNA-485-5p both inhibit proliferation and promote apoptosis in HCC cells. MiRNA-485-5p directly targete to the binding site of the MUC1 3'UTR and downregulated the expression of MUC1. Overexpression of MUC1 and miRNA-485-5p reverse the effect of miRNA-485-5p on the cell proliferation and apoptosis.

Conclusion MiRNA-485-5p inhibits the proliferation and facilitates the apoptosis by negatively regulating the expression of MUC1 in HCC cells.

Background

Hepatocellular carcinoma (HCC), one of the major malignant tumours, accounts for 75%-85% of liver tumours and approximately 12.5% of new malignant tumours in China per year [1, 2]. In 2018, approximately 841100 new cases of liver cancer were diagnosed; approximately 786600 patients die from liver cancer worldwide every year, and more than 50% of new cases and deaths occur in China [1, 3]. The incidence and mortality rates of liver cancer are decreasing year by year due to advances in diagnosis and therapy [4]. However, the 5-year survival rate of patients with liver cancer is only 30% [2]. Therefore, it is necessary to further research liver cancer to facilitate diagnosis and treatment.

With the in-depth study of tumorigenesis mechanisms, increasing evidence has shown that miRNAs are closely related to the development, prevention and treatment of various diseases, including cancers. miRNAs, small endogenous non-coding RNA with 20–24 nucleotides, are involved in post-transcriptional regulation of gene expression as thay affect both the stability and translation of mRNAs and play an important role in regulating cellular processes, especially cell development and tumorigenesis. Among them, miRNA-485-5p is located at 14q32.31 of the human genome. The differential expression of miRNA-485-5-p has been proven to participate in the development and prognosis of various cancers. MiRNA-485-5p is downregulated in the glima and involved in the progression of glioma, and this is mediated by an interaction with LINC00467 [5]. Moreover, miRNA-485-5 inhibits glioma cell proliferation and invasion by

targeting TPD52L2 [6]. In acute myeloid leukaemia, miRNA-485-5p suppresses the proliferation by antagonizing SALL4 [7]. MiRNA-485-5p promotes chemotherapeutic-induced cell death by downregulating PAK1 in oral tongue squamous cell carcinoma [8]. MiRNA-485-5p inhibites the proliferation and invasion of oesophageal cancer cells by antagonizing P-linked N-acetylglucosamine transferase [9].

MUC1 is located at 1q22 of the human genome and is expressed in throughout the body, but its expression is low in liver tissue. Mucin encodes a membrane-bound O-glycosylated protein that plays an essential role in forming protective mucous barriers on epithelial surfaces. Aberrant expression and intracellular localization, or changes in glycosylation of protein, have been associated with carcinomas. A large number of studies have shown that MUC1 is aberrantly expressed in many cancers and could be a biomarker for the prognosis and diagnosis of cancers, such as lung cancer [10], ovarian cancer [11], breast cancer [12], cervical cancer [13], and gastric cancer [14]. Studies have shown that antibodies targeting MUC1 peptides can prevent metastasis, counteract immunosuppression and serve as peptide vaccines for the treatment of cancers [15–17]. A researcher showed that the expression of MUC1 was related to progression, vascular invasion and cancer immune surveillance in liver cancer [18]. Another study showed that miRNA-485-5p inhibits the progression of breast cancer cells by negatively regulating MUC1 [12]. In HCC, it remains unclear whether miRNA-485-5p regulates the progression in a manner similar to that in breast cancer.

In this study, we discovered that the expression of miRNA-485-5p was downregulated and the expression of MUC1 was upregulated in HCC. The correlation analysis showed that the expression of miRNA-485-5p was negatively correlated with the expression of MUC1. Molecular mechanism studies indicated that miRNA-485-5p inhibited cell proliferation and facilitated apoptosis by antagonizing the expression of MUC1.

Methods

Clinical samples

The HCC and adjacent tissues were collected from 58 diagnosed HCC patients who had not received chemotherapy, rediation therapy or other anticancer treatment before undergoing surgical resection at the First pepole's Hospital of Huaihua from June 2019 to December 2019. The project was approved by the Medical Research Ethics Committee of Hunan University of Medicine and adhered to the principles in the Declaration of Helsinki. All patients signed informed consent at the time of surgery.

Cell culture

All cells were purchased from ATCC and cultured at 37 °C in 5% CO_2 incubator with saturate humidity. The SMMC7721 cell line was cultured in RPMI-1640 (Gibco, USA) and other HCC cells were cultured in DMEM (Gibco, USA). All medium were supplemented with 10% FBS (Gibco, USA) and 1% pencillin-streptomycin (Sigma, USA).

Cell transfection

MiRNA-485-5p minic, control minic (Ctrol minic), inhibitors, siRNA cotrol (si-Ctrol) and siRNA of MUC1 (si-MUC1) were synthesized by GenePharma (Shanghai, China). Small molecule RNA (minics, inhibitors, si-Ctrol and si-MUC1) were transfected into SMMC7721 cells using Lipofectamine^{R}3000 reagent in accordance with manufacturer's protocol.

qPCR

Total RNA was isolated from cells by the TransZol Up Plus RNA Kit (TRANSGEN, China). Accordance with the manufacturer's protocol, cDNA was synthesized by the Transcriptor First Strand cDNA Synthesis Kit (Roche, Switzerland). The expression of miRNA-485-5p and MUC1 were analyzed using the Bsetar^{R} SybrGreen qPCR Mastermix (DBI, GER) in according to the manufacturer's protocol by StepOnePlus^{TM} real-time PCR system (AB, USA). 18S, U6 were selected as the internal reference of MUC1 and miRNA-485-5p, respectively. All primers were synthesized by Sangon (Shanghai, China) and shown in Table 1.

Western blot

Total proteins from patients with HCC or cells were extracted using RIPA lysis buffer (P0013B, Beyotime, China) and quantified by BCA protein assay kit (PA101-01, Biomed, China) in according to the manual's protocol. Total proteins (15 μg/well) were separated by 8% SDS-PAGE, tansferred onto a PVDF membrane (IPVH00010, Millipore, Germany), blocked with 5% skim milk (D8340, Solarbio, China) at room temperature (RT) for 1-2 h. The membranes were incubated with anti-MUC1 (ab181133, abcam, USA) or anti-β-actin (ab179467, abcam, USA) at 4 ℃ overnight, and then incubated with the anti-rabbit IgG (ab6721, abcam, USA) at RT for 1 h. Finally, the MUC1 and β-actin proteins were visualized by chemiluminescence.

Immunohistochemistry

Sections from paraffin embedded blocks of HCC tissue were cut at 1-2 μm thickness and dewaxed. Then, heat-induced antigen retrieval of sections was performed in an autoclave at 120 ℃ for 10 min. Nonspecific antigen was blocked by 2% bovine serum albumin. The sections were incubated with an anti-MUC1 at 4 ℃ overnight, followed by incubation with a horseradish peroxidase (HRP)-conjugated anti-rabbit IgG at RT for 30 min. The sections were incubated with 3,3N-Diaminobenzidine tertrahydrochloride for a few seconds and counterstained with hematoxylin. Finally, the sections were viewed under a microscope (Carl Zeiss, GER).

Proliferation assay

The SMMC7721 and Huh7 cells were seeded into 96-well paltes at the density of 1 x 10^4 cells/well and transfected small molecule RNA. It was considered as 0 h when cells were attached. The cell viability of HCC cells was measured with the cell count kit-8 (CCK-8, Dojindo, Japan) according to the instruction protocol at indicated time respectively.

Apoptosis assay

After 8 h of transfection, apoptosis was detected in according to the manufacturer's protocol by flow cytometry (BD FACSCalibur, USA) using Annexin V-FITC kit (Dojindo, Japan). Briefly, the cells were suspended in 1x Annexin V binding solution at the concentration of $1x10^6$ cells/ml. Then, the 100 µl of cells suspension were incubated with Annexin V and PI for 15 minutes in the dark. After dilution with 400µl 1x Annexin V binding solution, the apoptosis of cells was analyzed by flow cytometry.

Dual luciferase assay

The MUC1 3' UTR wild-type (WT) or mutant (MT) binding to miRNA-485-5p were synthesized by Songon (Shanghai, China) and inserted into the pGL3 luciferase reporter vectors (Promega, USA) to generated pGL3-MUC1-WT and pGL3-MUC1-MT plamids. The pGL3-MUC1 vector (pGL3-MUC1-WT or pGL3-MUC1-MT) was co-transfected with miRNA-485-5p or control minic (Ctrol minic) into SMMC7721 cells using Lipofectamine^{R}3000 reagent in accordance with manufacturer's protocol. After 48 h of transfection, luciferase activity was analyzed by SpectraMax® M5 Multi-Mode microplate reader (MD, USA).

Statistical analysis

All data from three independent experiments were presnted as the mean ± standard deviation and processed by SPSS23.0 and GraphPad Prism 7.0. The student's *t* test was used for analyzing difference between two groups. The difference of among groups was analyzed with two-way ANOVA. The Spearman correlation analysis was performed to analyze the correlation between expression of miRNA-485-5p and expression of MUC1 in the patients with HCC. The p value <0.05 was considered as statistically significant.

Results

1. Differential expression of miRNA-485-5p and MUC1 in HCC

The expression of miRNA-485-5p and MUC1 in patients with HCC was analysed by qPCR. The results showed that the expression of miRNA-485-5p in HCC was significantly lower than that in adjacent HCC tissues ($p<0.0001$) (Figure 1A), while, the relative expression of MUC1in HCC was remarkably higher than that in adjacent tissues ($p<0.0001$) (Figure 1B). Then, the correlation analysis between miRNA-485-5p and MUC1 showed that the expression of miRNA-485-5p was negatively correlated with the expression of MUC1 in HCC (Figure 3C). Furthermore, the expression of MUC1 protein in patients with HCC was analysed by immunohistochemistry and western blot. The results showed that MUC1 was upregulated in HCC (Figure 1D & 1E).

2. Silencing MUC1 inhibits the proliferation and promotes the apoptosis of HCC cells

To investigate the effect of MUC1 on HCC cells, MUC1 was knocked down in HCC cells, and the proliferation and apoptosis of HCC cells were analysed. First, the expression of miRNA-485-5p and MUC1

was detected by qPCR. The expression of miRNA-485-5p in HCC cells was distinctly lower than that in liver cells (LO2) ($p<0.0001$) (Figure 2A), and MUC1 expression in HCC cells was distinctly higher than that in LO2 cells ($p<0.001$) (Figure 2B). Due to their expression of miRNA-485-5p and MUC1, SMMC7721 and Huh7 cells were used for further research.

Then, SMMC7721 and Huh7 cells were transfected with siRNA control (si-Ctrol) or siRNA-MUC1 (si-MUC1). The relative levels of MUC1 mRNA and protein were detected by qPCR and western blot, respectively. The results showed that the expression of MUC1 mRNA and protein was downregulated in SMMC7721 and Huh7 transfected si-MUC1 compared to those transfected with control (Figure 2C and 2D).

Further, cell viability and apoptosis were analysed by CCK-8 assay and flow cytometry assay, respectively. The relative OD value in SMMC7721 cells transfected with si-MUC1 was lower than that in those transfected with si-Ctrol, which suggested that silencing MUC1 inhibited the proliferation of SMMC7721 cells (Figure 2E). The effects of si-MUC1 transfection were similar in Huh7 and SMMC7721 cells (Fiuge 2E). The flow cytometry assay showed that apoptosis in SMMC7721 and Huh7 cells transfected with si-MUC1 was higher than that in cells transfected with si-Ctrol (Figure 2F), which revealed that knockdown MUC1 promoted apoptosis in HCC cells.

3. Overexpression of miRNA-485-5p inhibits the proliferation and promotes apoptosis of HCC cells

To explore whether miRNA-485-5p regulated the biological behaviour of HCC cells, including proliferation and apoptosis, HCC cells that overexpressed miRNA control (OE-Ctrol) and miRNA-485-5p (miRNA-485-5p mimic) were constructed. The expression of miRNA-485-5p was detected by qPCR. The relative expression of miRNA-485-5p in the SMMC7721 and Huh7 cells transfected with miRNA-485-5p mimic was higher than that in those transfected with OE-Ctrol (Figure 3A). Then, the CCK-8 assay showed that the relative OD value in miRNA-485-5p-overexpressing SMMC7721 and Huh7 cells was significantly lower than that in OE-Ctrol cells, which suggested that overexpressing miRNA-485-5p inhibited the proliferation of HCC cells (Figure 3B). The flow cytometry assay showed that the apoptosis rate of SMMC7721 and Huh7 cells overepxressing miRNA-485-5p was higher than that of OE-Ctrol cells, which indicated that miRNA-485-5p promoted the apoposis of HCC cells (Figure 3C).

4. MiRNA-485-5p directly targets MUC1

To investigate whether MUC1 was a direct target of miRNA-485-5p, bioinformatic analysis was performed. As shown in Figure 4A, some sites of the MUC1 3’ UTR were predicted to bind to miRNA-485-5p. Then, a luciferase reporter assay was performed to verify the interaction of the MUC1 3’ UTR with miRNA-485-5p. The results showed that luciferase activity in the cells co-transfected with miRNA-485-5p mimic and MUC1 3’ UTR MT was markedly reduced compared with that in the cells co-transfected with Ctrol mimic and MUC1 3’ UTR MT ($p<0.001$), while there was no significant difference between cells co-transfected with MUC1 3’ UTR MT and Ctrol minic or miRNA-485-5p ($p>0.05$) (Figure 4B). The expression of MUC1 was further detected by western blot of SMMC7721 cell lysates. Figure 4C shows that the

expression of MUC1 in the miRNA-485-5p group was significantly downregulated compared with that in the Ctrol mimic group. Compared with that in the Ctrol inhibitor group, the expression of MUC1 was significantly upregulated in the miRNA-485-5p group.

5. MiRNA-485-5p inhibits proliferation and promotes apoptosis by targeting MUC1 in HCC cells

To further explore whether miRNA-485-5p inhibited proliferation and promoted apoptosis by targeting MUC1 in HCC cells, a rescue experiment was performed in SMMC7721 cells. The relative expression of miRNA-485-5p and MUC1 mRNA was detected by qPCR. As shown in Figure 5A, the expression of miRNA-485-5p in the cells co-transfected with miRNA-485-5p mimic and OE-Ctrol was significantly increased compared with that in the cells co-transfected Ctrol mimic and OE-Ctrol ($p<0.0001$). Compared with the expression of MUC1 in cells co-transfected with Ctrol mimic and OE-Ctrol, MUC1 expression was markedly downregulated in the cells co-transfected with miRNA-485-5p mimic and OE-Ctrol cells ($p<0.01$). However, the expression of MUC1 in the cells co-transfected with miRNA-485-5p and OE-MUC1 was upregulated compared with that in cells co-transfected miRNA-485-5p mimic and OE-Ctrol ($p<0.01$). Then, the proliferation of SMMC7721 cells was analysed by CCK-8 assay, and apoptosis was detected by flow cytometry. Compared with that in cells co-transfected with Ctrol mimic and OE-Ctrol, the cell viability was markedly decreased, and the apoptosis rate was significantly increased in cells co-transfected with miRNA-485-5p mimic and OE-Ctro ($p<0.05$). Compared with that in the cells co-transfected with miRNA-485-5p mimic and OE-Ctrol, cell viability was significantly restored, and apoptosis was markedly decreased in cells co-transfected with miRNA-485-5p mimic and OE-MUC1 ($p<0.01$), which indicated that MUC1 rescued miRNA-485-5p-induced cell viability and apoptosis (Figure 5B and 5C).

Discussion

In this study, we found that the expression of miRNA-485-5p was downregulated; while the expression of MUC1 was upregulated in HCC tissues and HCC cell lines compared to normal controls. We concluded that the expression of miRNA-485-5p in HCC negatively correlated with the expression of MUC1 by correlation analysis. Dual luciferase analysis further revealed that MUC1 was a direct target gene of miRNA485-5p in HCC cells, which was consistent with the results reported by Wang et al [12].

The abnormal expression of MUC1 is associated with cancer progression [19] and can serve as a biomarker for the diagnosis of cancers [20, 21]. Furthermore, MUC1 can be used as a target gene for the treatment of cancers [17]. It is well known that cells in cancer tissue are heterogeneous and characterized by abnormal proliferation and reduced apoptosis. Cell proliferation was significantly inhibited, and cell apoptosis was dramatically promoted in SMMC7721 and Huh7 cells overexpressing si-MUC1, which showed that silencing MUC1 inhibited cell proliferation and promoted apoptosis. This suggests that the excessive growth of HCC may be due to the upregulation of MUC1 in HCC tissue, consistent with the research of Yuan et al [22].

Studies have shown that the expression of miRNA-485-5p is abnormal in the majority of tumours and is related to the risk of developing cancer [23]. It has been shown that the expression of miRNA-485-5p is decreased in breast cancer [12]. In our study, proliferation was reduced, and cell apoptosis was facilitated in HCC cells transfected with miRNA-485-5p mimic or si-MUC1. This result was consistent with Wang's research [12].

Through bioinformatic and dual luciferase assays, the targeting relationship between miRNA-485-5p and the binding site of the MUC1 3'UTR was verified in HCC cells. Further experiments showed that the expression of MUC1 was negatively regulated by miRNA-485-5p. The rescue experiment showed that the overexpression of MUC1 eliminated the inhibitory effects of miRNA-485-5p on proliferation and the promoting effects of miRNA-485-5p on apoptosis in HCC cells. In summary, the expression of miRNA-485-5p was downregulated, while the expression of MUC1 was upregulated in HCC tissue compared to nomal tissue. MiRNA-485-5p inhibited the proliferation and promoted apoptosis of HCC cells by targeting MUC1. However, the specific molecular mechanism need to be further investigated. MiRNA-485-5p plays an important role in the metabolism of carcinogens [24]. In the early stages of HCC development, it is possible that the expression of miRNA-485-5p is decreased, which can reduce or abrogated ability to metabolize carcinogens, thereby aggravating the progression of HCC.

Conclusions

In summary, in this study, we found that miRNA-485-5p can inhibit proliferation and promote apoptosis by targeting MUC1 in HCC cells. Therefore, miRNA-485-5p serves as a new biomarker for diagnosis of HCC, and miRNA-485-5p-MUC1 axis could be a potential target for HCC medical interventions.

Abbreviations

HCC: hepatocellular carcinoma; HRP: horseradish peroxidase; WT: wild-type; MT: mutant

Declarations

Ethical approval and consent to participate

All human tissues were approved by the Medical Research Ethics Committee of Hunan University of Medicine and adhered to the principles in the Declaration of Helsinki. All patients signed informed consent at the time of surgery.

Consent for publication

We confirm that all the listed authors have approved the publication of this manuscript.

Availability of data and materials

All data, analyzed the results of this article, are included in the article.

Competing interest

The authors declare that they have no competing interests.

Funding

This project was supported by PhD Research Startup Foundation of Hunan University of Medicine, China (No. 2018-01), Natural Science Foundation of Hunan Province, China (No. 2017JJ2200), and Scientific Research Fund of Hunan Provincial Education Department, China (No. 19A355).

Author contributions

ML, DZ, XP and LW conceived and designed the experiments, and checked and revised the manuscript. ML, DZ, XP, PL, FY, HL, XZ and FL performed all experiments. FY collected the HCC tissues. ML and XP analyzed the data in manuscript. ML and DZ writed the manuscript. All authors approved the manuscript and the ordering of authors.

Acknowledgements

Not applicable.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016; 66: 115-32.
2. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Niksic M, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 2018; 391: 1023-1075.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394-424.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020; 70: 7-30.
5. Jiang XH, Liu YY. LINC00467 promotes proliferation and invasion in glioma via interacting with miRNA-485-5p. Eur Rev Med Pharmacol Sci. 2020; 24: 766-772.
6. Yu J, Wu SW, Wu WP. A tumor-suppressive microRNA, miRNA-485-5p, inhibits glioma cell proliferation and invasion by down-regulating TPD52L2. Am J Transl Res. 2017; 9: 3336-3344.
7. -L-Wang W, Wang HR, Ji WG, Guo SL, Li HX, Xu XY. MiRNA-485-5p suppresses the proliferation of acute myeloid leukemia via targeting SALL4. Eur Rev Med Pharmacol Sci. 201;. 23: 4842-4849.

8. Lin XJ, He CL, Sun T, Duan XJ, Sun Y, Xiong SJ. hsa-miR-485-5p reverses epithelial to mesenchymal transition and promotes cisplatin-induced cell death by targeting PAK1 in oral tongue squamous cell carcinoma. Int J Mol Med. 201; 40: 83-89.
9. Han DL, Wang LL, Zhang GF, Yang WF, Chai J, Lin HM, Fu Z, et al. MiRNA-485-5p, inhibits esophageal cancer cells proliferation and invasion by down-regulating O-linked N-acetylglucosamine transferase. Eur Rev Med Pharmacol Sci. 2019; 23: 2809-2816.
10. Arroyo M, Larrosa R, Gómez-Maldonado J, Cobo MÁ, Claros MG, Bautista R. Expression-based, consistent biomarkers for prognosis and diagnosis in lung cancer. Clin Transl Oncol. 2020; doi: 10.1007/s12094-020-02328-4.
11. Gong G, Lin T, Yuan Y. Integrated analysis of gene expression and DNA methylation profiles in ovarian cancer. J Ovarian Res. 2020; 13: 30.
12. Wang X, Zhou X, Zeng F, Wu X, Li H. miR-485-5p inhibits the progression of breast cancer cells by negatively regulating MUC1. Breast Cancer. 2020; doi: 10.1007/s12282-020-01075-2.
13. Zhang J, Wang L, Jiang J, Qiao Z. Elevation of microRNA-512-5p inhibits MUC1 to reduce radioresistance in cervical cancer. Cell Cycle. 2020; 19: 652-665.
14. Ge Y, Ma G, Liu H, Lin Y, Zhang G, Du M. MUC1 is associated with TFF2 methylation in gastric cancer. Clin Epigenetics. 2020; 12: 37.
15. Mohit E, Hashemi A, Allahyari M. Breast cancer immunotherapy: monoclonal antibodies and peptide-based vaccines. Expert Rev Clin Immunol. 2014; 10: 927-61.
16. Li M, Wang Z, Yan B, Yin X, Zhao Y, Yu F, et al. Design of a MUC1-based tricomponent vaccine adjuvanted with FSL-1 for cancer immunotherapy. Medchemcomm. 2019; 10: 2073-2077.
17. Yu H, Ye C, Li J, Pan C, Lin W, Chen H, et al. An altered HLA-A0201-restricted MUC1 epitope that could induce more efficient anti-tumor effects against gastric cancer. Exp Cell Res. 2020; doi: 10.1016/j.yexcr.2020.111953.
18. Pascut D, Krmac H, Gilardi F, Patti R, Calligaris R, Crocè LS, et al. A comparative characterization of the circulating miRNome in whole blood and serum of HCC patients. Sci Rep. 2019; 9: 8265.
19. Díaz Del Arco C, Garré P, Molina Roldán E, Lorca V, Cerón Nieto MÁ, Fernández Aceñero MJ. MUC1 expression in colorectal carcinoma: Clinicopathological correlation and prognostic significance. Rev Esp Patol. 2018; 51: 204-209.
20. Ma S, An F, Li LH, Lin YY, Wang J. Expression of Mucin 1 in salivary gland tumors and its correlation with clinicopathological factors. J Biol Regul Homeost Agents. 2019; 33: 563-569.
21. Li X, Xu Y, Zhang L. Serum CA153 as biomarker for cancer and noncancer diseases. Prog Mol Biol Transl Sci. 2019: 162: 265-276.
22. Yuan H, Wang J, Wang F, Zhang N, Li Q, Xie F, et al. Mucin 1 gene silencing inhibits the growth of SMMC-7721 human hepatoma cells through Bax-mediated mitochondrial and caspase-8-mediated death receptor apoptotic pathways. Mol Med Rep. 2015; 12: 6782-8.

23. Lu H, Wang C, Xue L, Zhang Q, Luh F, Wang J, et al. Human Mitotic Centromere-Associated Kinesin Is Targeted by MicroRNA 485-5p/181c and Prognosticates Poor Survivability of Breast Cancer. J Oncol. 2019; 2019: 2316237.
24. Sutliff AK, Shi J, Watson CJW, Hunt MS, Chen G, Zhu HJ, et al. Potential Regulation of UGT2B10 and UGT2B7 by miR-485-5p in Human Liver. Mol Pharmacol. 2019; 96: 674-682.

Tables

Table 1. The sequences of all primers in this study

Nanes	Sequences (5′-3′)
miRNA-485-5p	Forward: CCAAGCTTCACCCATTCCTAACAGGAC
	Reverse: CGGGATCCGTAGGTCAGTTACATGCATC
U6	Forward: CTCGCTTCGGCAGCACA
	Reverse: AACGCTTCACGAATTTGCGT
MUC1	Forward: AGTGCTTACAGTTGTTACGGGT
	Reverse: AGTAGTCGGTGCTGGGATCT
18S	Forward: AGAAACGGCTACCACATCCA
	Reverse: CACCAGACTTGCCCTCCA

Figures

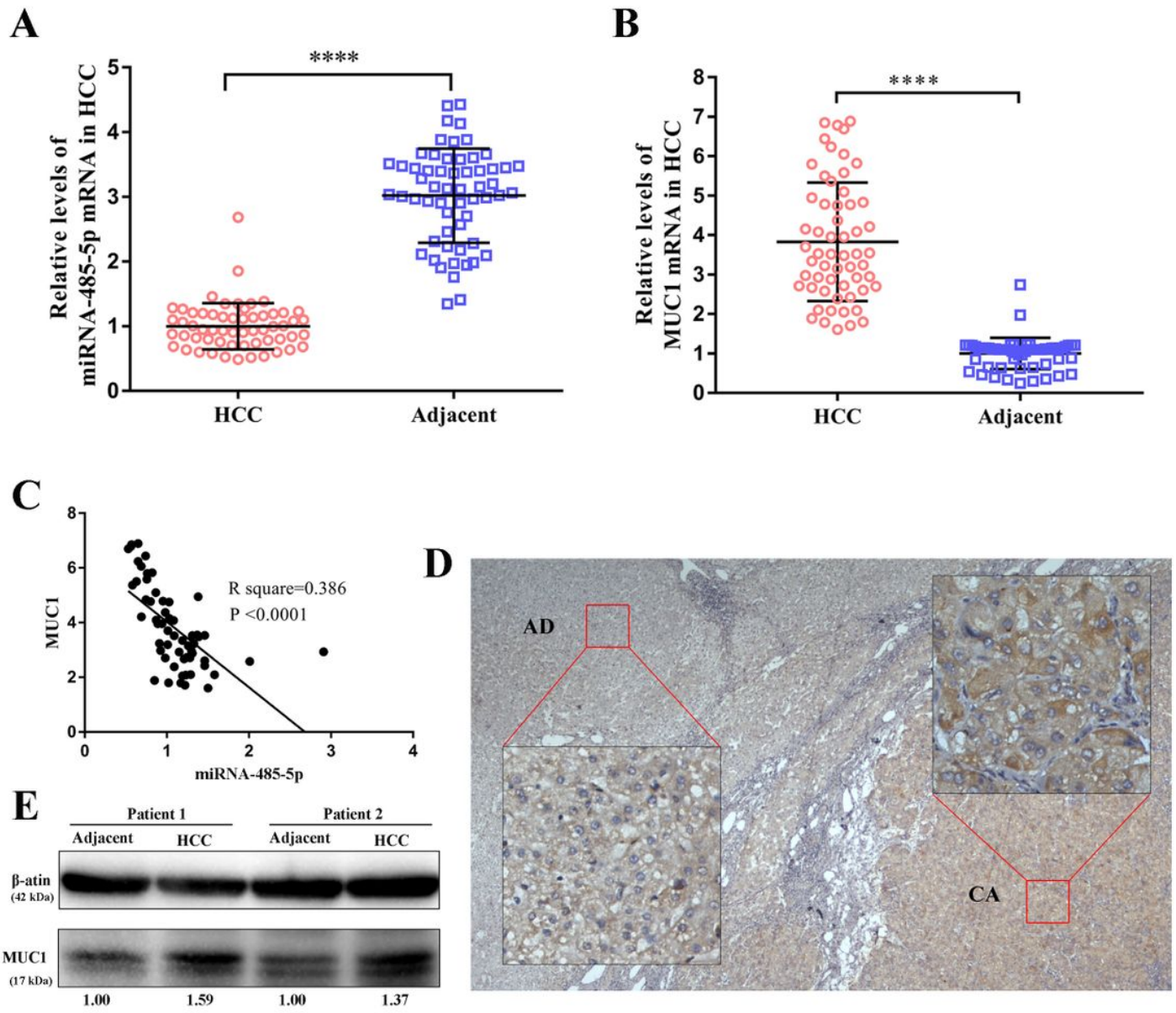

Figure 1

The expression of miRNA-485-5p and MUC1. The expression of miRNA-485-5p (A) and MUC1 (B) in the patients with HCC was detected by qPCR (n=58, t-test, ****p<0.0001). C. The correlation between miRNA-485-5p and MUC1 was analyzed. The expression of MUC1 in patient with HCC was measured by immunohistochemistry (D) and western blot (E). CA: liver cancer, AD: adjacent.

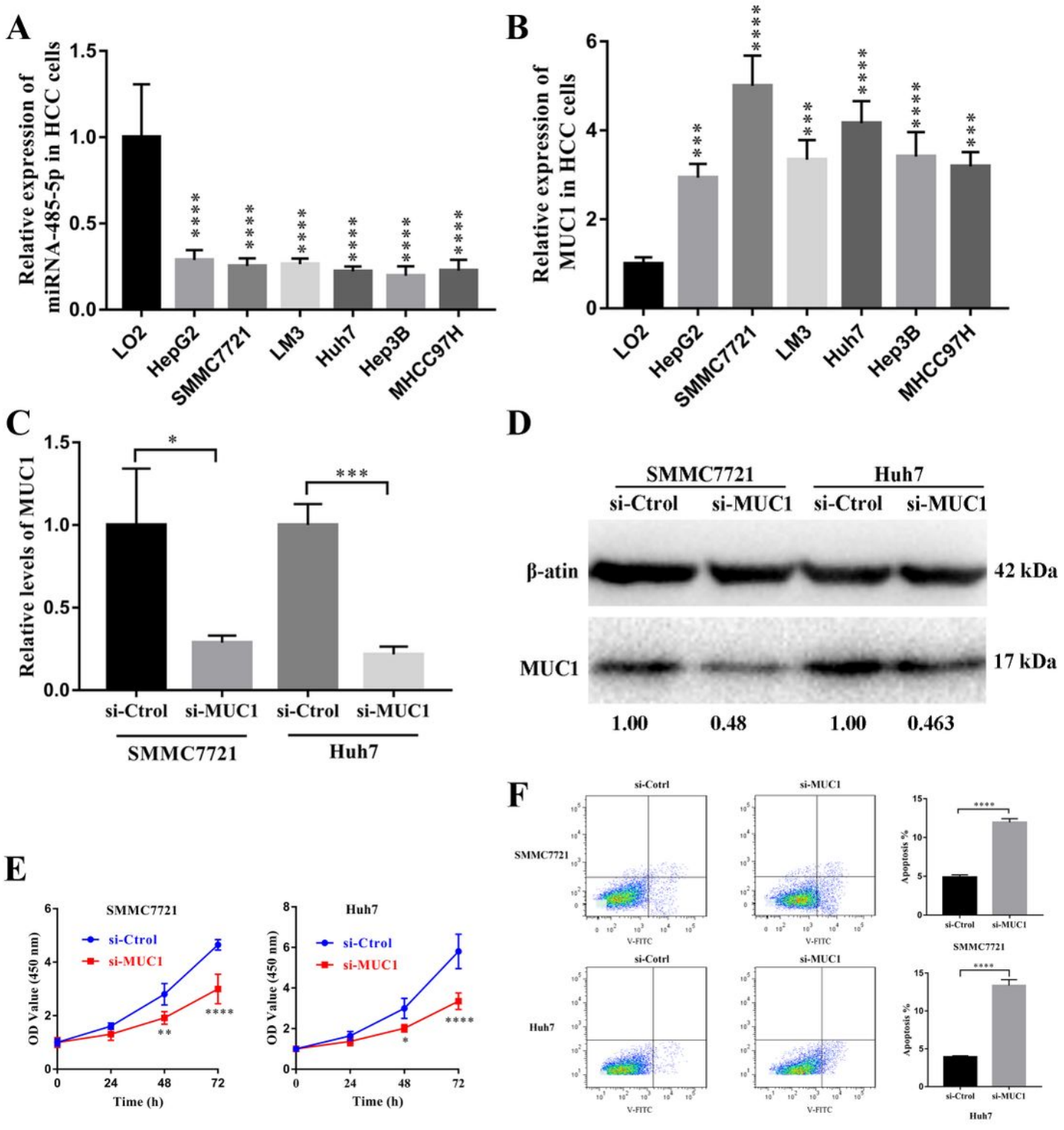

Figure 2

Silencing MUC1 inhibits the proliferation and promote the apoptosis in HCC cells. The relative expression of miRNA-485-5p (A) and MUC1 (B) was detected by qPCR in HCC cells (t-test, ***p<0.001, ****p<0.0001). C. The relative expression of MUC1 mRNA was analyzed by qPCR in SMMC7721 and Huh7 cells overexpressed si-MUC1 (t-test, *p<0.05, ***p<0.001). D. The expression of MUC1 was displayed by western blot in the SMMC7721 and Huh7 overexpressed si-MUC1. E. The cell viability of SMMC7721 (left)

and Huh7 (right) overexpressed si-MUC1 was analyzed by CCK-8 assay (tow-way ANOVA, *p<0.05, **p<0.01, ****p<0.0001). F. The apoptosis of SMMC7721 (top) and Hu7 (bottom) overexpressed si-MUC1 by flow cytometry assay (t-test, ****p<0.0001).

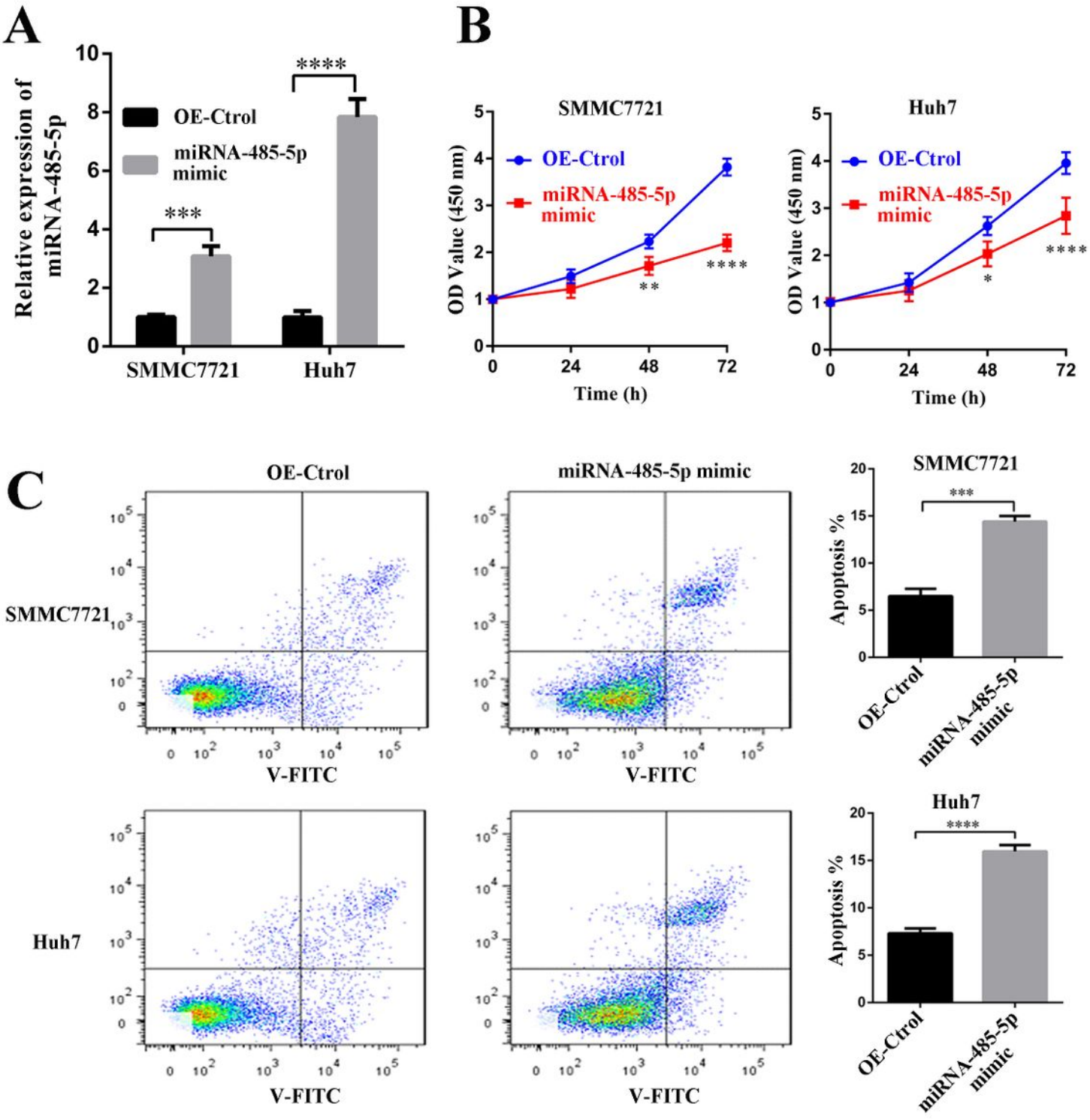

Figure 3

Overexpression of miRNA-485-5p inhibits the proliferation and promote the apoptosis in SMMC7721 and Huh7 cells. A. The relative expression of miRNA-485-5p was measured by qPCR in SMMC7721 and Huh7

overexpressed miRNA-485-5p (t-test, ***p<0.001, ****p<0.0001). B. The cell viability of SMMC7721 (left) and Huh7 (right) overexpressed miRNA-485-5p was analyzed by CCK-8 assay (tow-way ANOVA, *p<0.05, **p<0.01, ****p<0.0001). C. The apoptosis of SMMC7721 (top) and Hu7 (bottom) overexpressed miRNA-485-5p by flow cytometry assay (t-test, ***p<0.001, ****p<0.0001).

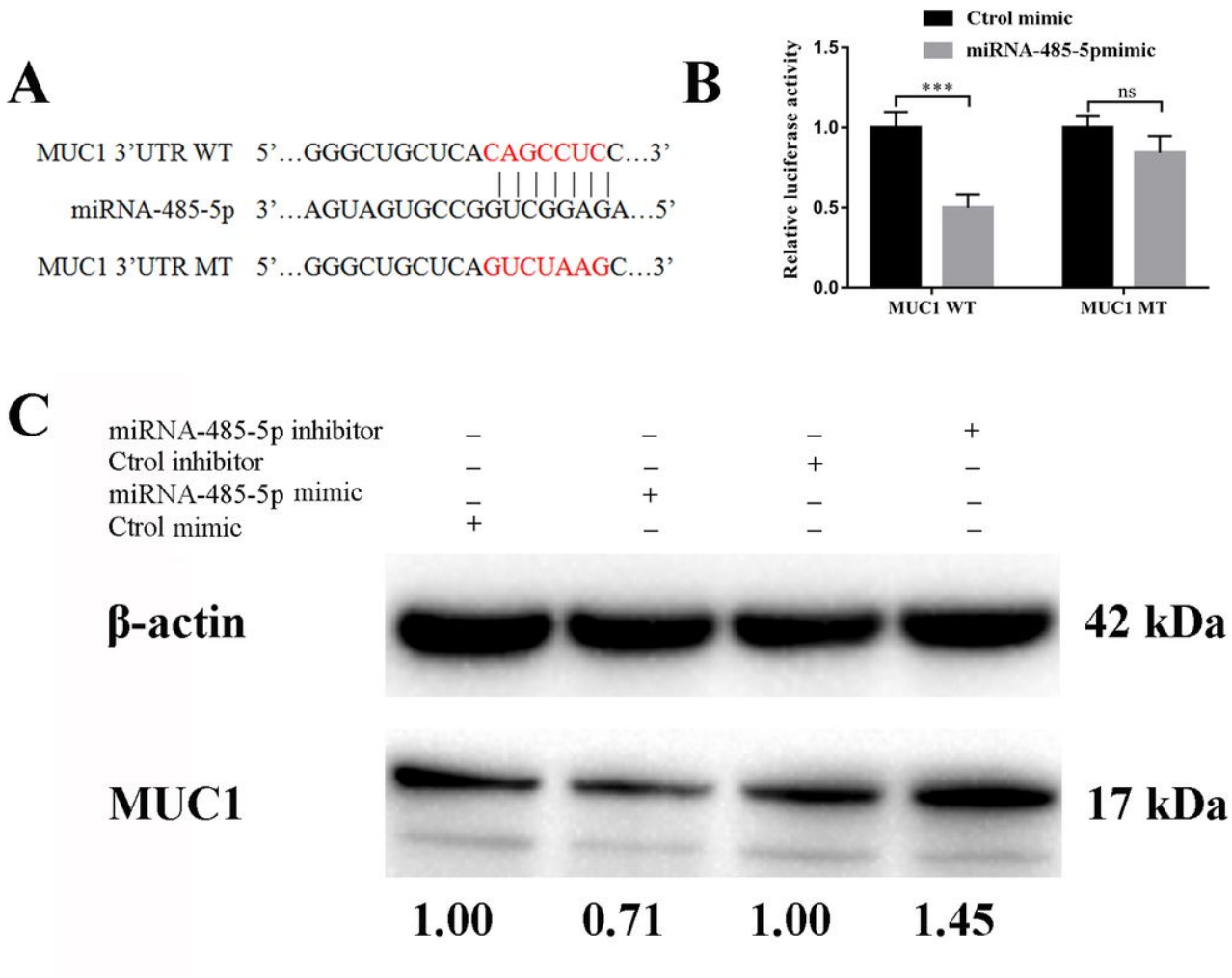

Figure 4

MiRNA-485-5p directly targets MUC1. A. The sites of MUC1 3' UTR were binded by miRNA-485-5p. B. The dual luciferase assay was prefomed to verify the directly target ralationship miRNA-485-5p and MUC1 (t-test, ***p<0.001, ns p>0.005). C. The expression of MUC1 was detected by western blot in the Ctrol mimic, miRNA-485-5p mimic, Ctrol inhibitor and miRNA-485-5p inhibitor (+: presence, -: absence).

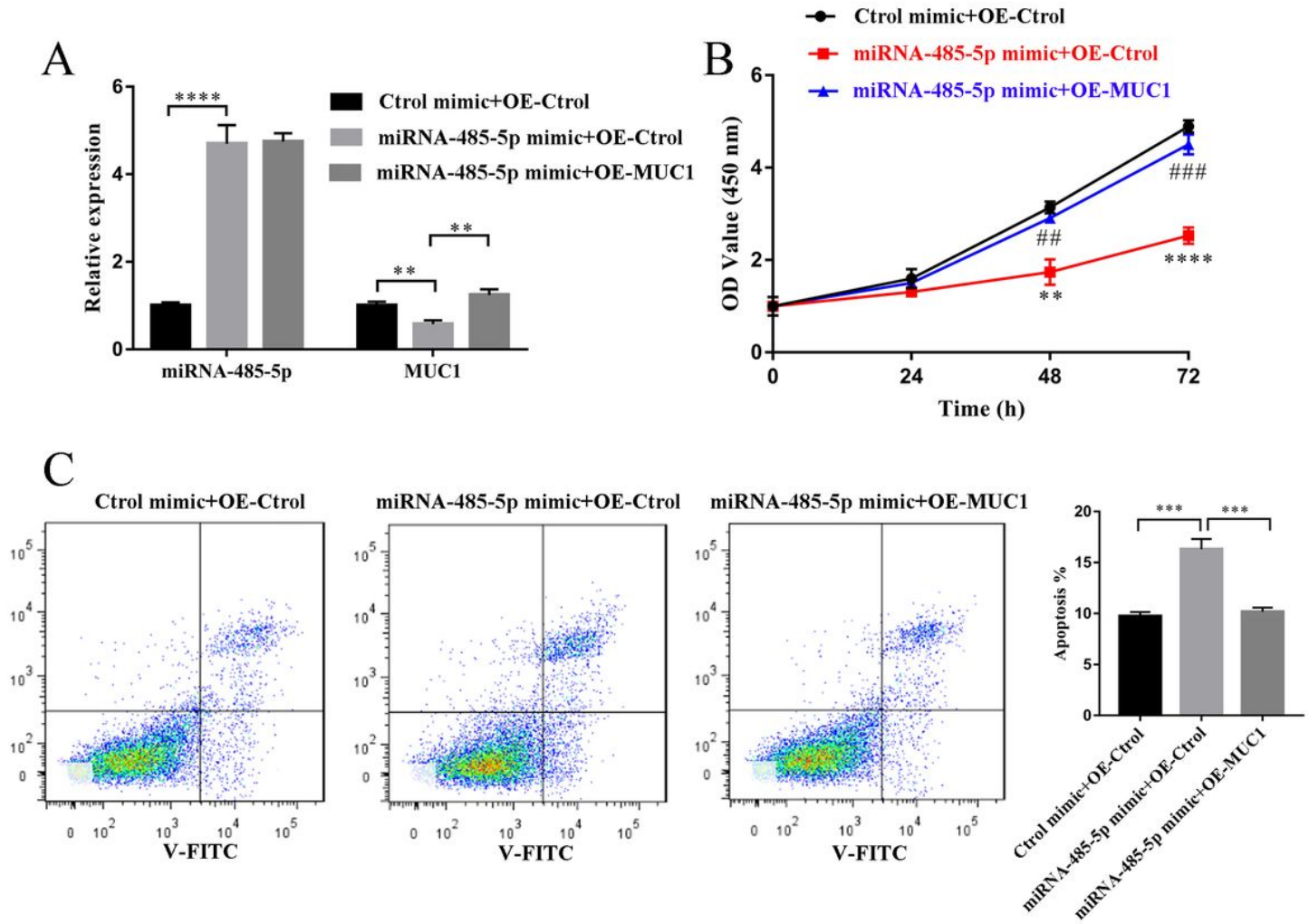

Figure 5

MiRNA-485-5p inhibit the proliferation and promote the apoptosis by targeting MUC1 in SMMC7721 cells. A. The relative expression of miRNA-485-5p and MUC1 was detected by qPCR in the indicated groups (t-test, **p<0.01, ****p<0.0001). B. The cell proliferation was analyzed by CCK-8 assay in the indicated groups ((tow-way ANOVA, **, ##p<0.01, ###p<0.001, ****p<0.0001). C. The apoptosis was analyzed by flow cytometry in the indicated groups (t-test, ***p<0.001).