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Silencing MRP7 Gene by siRNA Reversed Multidrug Resistance in Hepatocellular Carcinoma Resistant Cell Line HepG2/ADM and SMMC7721/ADM

Qing Chen[1], Fan Hu[2], Fan Zhang[1], Jie Zhou[2]*

[1]*Department of Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[2]*Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, No.13 Hangkong Road,Wuhan 430030, China*

Abstract

Objective: To investigate the effect on silencing MRP7 gene by siRNA reversed multidrug resistance in hepatocellular carcinoma resistant cell line SMMC7721/ADMand HepG2/ADM.

Methods: SMMC7721 and HepG2 cells were transfected with different gradient concentrations of doxorubicin (ADM) to produce HepG2/ADM and SMMC7721/ADM. MRP7-siRNA was transfected into HepG2/ADM and SMMC7721/ADM cells by lipofectamine 2000 liposomes. Real-time fluorescent quantitative RT-PCR was used to detect MRP7 mRNA expression in each group. Western blot was used to detect the expression of MRP7 protein. Transwell Invasion Assay and Flow Cytometry were used to detect the influence on the invasion ability and apoptosis of hepatoma cells after silencing MRP7 Gene.

Results: MTT assay revealed that the IC50 values and RI of SMMC7721/ADM and HepG2/ADM cells were decreased after treatment with siRNA. The mRNA expression of MRP7 was significantly decreased in SMMC7721/ADM and HepG2/ADM cells after siRNA transfection. Compared with the expression of parental cells, MRP7 protein expressions were apparently decreased in SMMC7721/ADM and HepG2/ADM cells. Flow cytometry showed that silencing MRP7 gene may result in a significantly higherrate of apoptosis of HepG2/ADM and SMMC7721/ADM cells. Transwell assays showed that silencing MRP7 gene significantly reduced the invasive potential of SMMC7721/ADM and HepG2/ADM cells.

Conclusion: After silencing MRP7 gene in SMMC7721/ADM and HepG2/ADM cells by siRNA, the sensitivity of the cells to chemotherapeutic drugs was significantly increased and can partially reverse the drug resistance of the cells to chemotherapeutic drugs.

Keywords: Liver neoplasms; Experimental; RNA interference; Multidrug resistance-associated proteins

Introduction

With the continuous changes of people's lifestyle and diet, the new incidence and the mortality of liver cancer also increased year by year. Primary liver cancer is one of the world's highest fatality, the highest mortality in all the malignant tumors [1,2].

According to the International Agency for Research on Cancer (IARC), China has a high incidence of HCC with more than 560,000 new patients worldwide each year, of which about 550,000 die from liver cancer. However, only about 306,000 new cases of HCC in China are reported with about 300,000 deaths, accounting for 54.26% and 54.64% of the world's total respectively, ranking first in the world and causing major health threats and economic losses to our country [3]. There are many causes of primary liver cancer, mainly chronic hepatitis B and Hepatitis C virus infection and long-term exposure to aflatoxin and other toxic substances. In addition, according to the study, smoking, drinking, fluke disease, high-fat diet, poisoning and other factors also have some relevance with primary liver cancer [4-6].

Surgical resection of tumor lesions is the first choice of treatment for liver cancer; however, the proportion of postoperative recurrence of liver cancer is up to 60% and ranking in the first three causes of cancer death [7]. According to clinical statistics, some patients who cannot get surgical resection after postoperative recurrence of liver cancer can still get some curative effect by chemotherapy [8]. Although new chemotherapeutic drugs and chemotherapy regimens are being developed at present, the existence of Multidrug Resistance (MDR) affects the efficacy of chemotherapy for liver cancer and reduce the effect of chemotherapy and lead to liver cancer recurrence and metastasis, which is the current clinical problems to be solved [9,10].

Under the attack of various internal or external human or internal factors, tumor cells can establish an adaptive mechanism in the cell to deal with various strike processes and thus survive in the harsh environment inside and outside, which is also the reason that tumor cells respond to chemotherapy drugs. The nature of drug resistance, the occurrence and maintenance of adaptation processes

***Corresponding author:** Jie Zhou, Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, No.13 Hangkong Road, Wuhan 430030, China, Tel: +86-027-83692608; E-mail: chentianyuzj@126.com

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are controlled by a complex network of molecules. In conclusion, in the gene expression level, there was profound significance in finding the molecular mechanism or molecules network change of drug resistance in tumor cells for us to thoroughly grasp the resistance mechanism, thereby reversing multi-drug resistance of tumor cells [11]. Currently, ABC-binding membrane transporters are considered as the most important and most studied resistance mechanisms. Among them, Multidrug Resistance-Related Protein (MRP) has gradually become a hot spot in recent research and its role in drug resistance is also increasingly recognized by people [12-14].

In previous studies, we established the SMMC-7721/ADM drug resistance liver cancer cell lines induced by continuous concentration gradient ADM and then down-regulated the MRP3 gene of SMMC-7721/ADM by using siRNA technology which increased the sensitivity to chemotherapeutic drugs and it proved that MRP3 gene was associated with multidrug resistance of liver cancer and it can be a therapeutic target for reversing the multidrug resistance of liver cancer. MRP7 is an ATP-dependent membrane transporter and is a member of the multidrug resistance protein family that is largely homologous in amino acid sequence to MRP3 and is also highly expressed in the liver, pancreas, biliary tract, adrenal, duodenum and colon. WetherMRP7 gene is also involved in multidrug resistance of liver cancer cells? This project intends to use liposome transfection technique to introduce specific siRNA gene fragment into HepG2/ADM and SMMC7721/ADM model and to down-regulate or close the MRP7 gene of drug-resistant cell line of hepatocellular carcinoma to increase the intracellular drug concentration and to promote the sensitivity of hepatoma cells to chemotherapy and improve the chemotherapy effect of liver cancer.

Materials and Methods

Material

Human hepatoma cell line HepG2, SMMC7721 was purchased from Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. The mouse anti-human MRP7 McAb was purchased from Taiwan Abnova Company. Lipofectamine 2000 liposomes were purchased from the United States Invitrogen Corporation. Trizol was purchased from the United States GIBCO company. Thiazolyl blue (MTT) kit was purchased from Shanghai Pfeiffer Biotechnology Co. ABI Prism 7300 real-time fluorescence quantitative PCR instrument was purchased from the United States ABI PRISM company.

Methods

Establish multidrug resistance hepatoma cell line HepG2/ADM and SMMC7721/ADM: Human hepatocellular carcinoma cells SMMC7721 and HepG2 were cultured inDMEM containing 10% fetal calf serum and cultured in a 37°C incubator with 5% CO_2 and saturated humidity. Different concentrations of doxorubicin (ADM) from 0.1 μg/ml to 2.0 μg/ml were added gradually to DMEM medium with 0.1 μg/ml of each gradient. We designated the cultured cells as HepG2/ADM and SMMC7721/ADM cellswhen ADM concentration was stable at 2.0 μg/ml.

MRP7-siRNA transfection: Take MRP7 gene as the interference target and use Thermo online design software to design MRP7-siRNA sequence. siRNA sequence: GGAAAGAAGUCGGGGCAUA. HepG2/ADM and SMMC7721/ADM cells were seeded in a 6-well plate at a concentration of 6 × 10 4 cells/well. After being cultured for 48 h, the cells were transfected with Lipofectamine 2000 liposomes. After being transfected with 5% CO_2 at 37°C for 4 h, the DMEM medium was replaced and cultured for 24 h.

Experimental grouping: The experiment was divided into HepG2 cell group (control group), HepG2/ADM cell group (drug-resistant group), HepG2/ADM cell group transfected with MRP7-siRNA (interference group) and andSMMC7721 cell group (control group), SMMC7721/ADM cell group (drug-resistant group), SMMC7721/ADM cell group transfected with MRP7-siRNA (interference group).

Detect mRNA expression levels of MRP7 gene by Real-time fluorescent quantitative RT-PCR: Six groups of cells were inoculated with 6-well plates respectively and then collected cells after continue to culture for 24 h. Trizol was used to extract the total RNA of the six groups of cells and 2 μg of RNA was reverse transcribed to synthesize cDNA. MRP7 primer sequence: upstream 5-ATCCTGGCGATCTACTTCCT-3, downstream 5-TACAGCTTCAGCACCTTGAT-3. Take 18SRNA primer as internal control. The conditions for PCR amplification were degeneration at 95°C for 10 min, 40 PCR cycles, extension at 72°C for 5 min and detection of fluorescence signal at 75°C and then draw a standard curve according the above data. The mRNA expression levels of MRP7 gene and 18S RNA were obtained. The homology analysis was performed using DNASIS software. The sequencing results were aligned with CLUSTALW software and phylogenetic tree was analyzed by MEGA software.

Detect MRP7 protein expression by Western-blot: The cells were processed and collected as described above. Total protein was extracted from each of the six groups of cells and 30 μg total protein was subjected to SDS-PAGE, low temperature constant pressure transfer film, 100 V 90 min; 30 min, blocked for 2 h at room temperature, added primary and secondary antibodies, visualized protein bands by ECL with GAPDH as internal reference and then made semi-quantitative analysis with Quantity One gray-scale analysis software.

Detect cell apoptosis before and afterMRP7-siRNA transfection by flow cytometry: HepG2 and SMMC7721 cells were seeded into 6-well plates and transfected with MRP7 siRNA for 48 h. Then, cells were digested with 0.25% trypsin followed by 1 mM EDTA buffer, and then fixed in 70% ethanol at 4°C. The apoptosis was assessed by flow cytometry using the Annexin V apoptosis detection kit.

Detect invasive ability before and afterMRP7-siRNA transfection by Transwell assay: At 24 h after transfection, cells were treated with 0.25% trypsin, centrifuged at 1000 rpm for 10 min, and then suspended in 1640 medium containing 10% fetal bovine serum. Cells were then plated as single-cell suspension in Transwell chambers (1×10^5 cells/well/200 μl) and maintained in 700 μl of 1640 medium containing 10% fetal bovine solution. Six groups of cells (experimental group and empty vector control group) were incubated at 37°C with 5% CO_2 for 48 h. Then, cells were fixed in formalin and examined under a microscope at low magnification. Three fields were randomly chosen. The number of cells per field was counted at high magnification.

Statistical analysis: Data were analyzed using the SPSS 20.0 software (SPSS, Chicago, IL, USA). Rank sum test was used to examine the differential expression of multidrug resistance proteins in hepatoma cells. All experiments were repeated 3 times, each performed in triplicate. The average value of each group was calculated. Student's t-test was used to compare the results between two groups; while Dunnett's test was performed among three or more groups. If the variances were inhomogeneous in Student's t-test, the results were analyzed using Welsh test. Data are expressed as mean +/− SEM. $P < 0.05$ indicated statistical significance.

Results

mRNA expression after silencing MRP7 gene in HepG2 and SMMC7721 cells

MRP7 mRNA expression was detected by qRT-PCR after silencing MRP7 gene in HepG2 and SMMC7721 cells. Silencing MRP7 gene significantly decreased MRP7 mRNA expression levels (Figure 1).

MRP7 protein level after silencing MRP7 gene in HepG2 and SMMC7721 cells

Western blot results showed that MRP7 protein was expressed in HepG2 cells (control group), HepG2/ADM cells (resistant group) and HepG2/ADM transfected MRP7- siRNA group (interference group). The expression of MRP7 protein in the control group, drug-resistant group and interference groupwere2501.26, 2838.22 and 925.35 respectively. The expression of MRP7 protein in the interference group was significantly lower than that in the drug-resistant group and the control group (Figure 2 and Table 1).

Western blot results showed that MRP7 protein was expressed in SMMC7721 cells (control group), SMMC7721/ADM cells (resistant group) and SMMC7721/ADM transfected MRP7-siRNA group (interference group). The expression of MRP7 protein in the control group, drug-resistant group and interference group were 1620.36, 1861.50 and 249.29 respectively. The expression of MRP7 protein in the interference group was significantly lower than that in the drug-resistant group and the control group (Figure 2 and Table 1).

Cell apoptosis after silencing MRP7 gene in HepG2/ADM and SMMC7721/ADM cells

The rates of late apoptosis of HepG2/ADM, HepG2/ADM NCsiRNA and HepG2/ADM MRP7siRNA were 0.98, 1.02 and 1.60 respectively. It can be seen that after silencing MRP7 gene, the proportion of late apoptotic cells was significantly increased (Figure 3 and Figures 4A-4C) (Table 2).

The rates of early apoptosis of HepG2/ADM, HepG2/ADM NCsiRNA and HepG2/ADM MRP7siRNA were 0.71, 0.52 and 0.53, respectively. It can be seen that after silencing MRP7 gene, the proportion of early apoptotic cells decreased slightly.

The total apoptosis rates of HepG2/ADM, HepG2/ADM NCsiRNA and HepG2/ADM MRP7siRNA were 1.69, 1.54 and 2.13, respectively. It can be seen that after silencing MRP7 gene, the total apoptosis of cells increased significantly.

The rates of late apoptosis of SMMC7721/ADM, SMMC7721/ADM NCsiRNA and SMMC7721/ADM MRP7siRNA were 2.35, 1.32 and 17.53, respectively. It can be seen that after silencing MRP7 gene, the proportion of late apoptosis was significantly increased (Figure 5 and Figures 6A-6C) (Table 3).

The rates of early apoptosis of SMMC7721/ADM, SMMC7721/ADM NCsiRNA and SMMC7721/ADM MRP7siRNA were 0.96, 0.65 and 7.56, respectively. It can be seen that after silencing MRP7 gene, the proportion of early apoptotic cells was significantly increased.

The total apoptosis of SMMC7721/ADM, SMMC7721/ADM NCsiRNA and SMMC7721/ADM MRP7siRNAs were 3.31, 1.97 and 25.09, respectively. It can be seen that after silencing MRP7 gene, the total apoptosis of cells increased significantly.

Silencing MRP7 gene resulted in a significantly higher rate of apoptosis of HepG2/ADM and SMMC7721/ADM cells, as determined by flow cytometry.

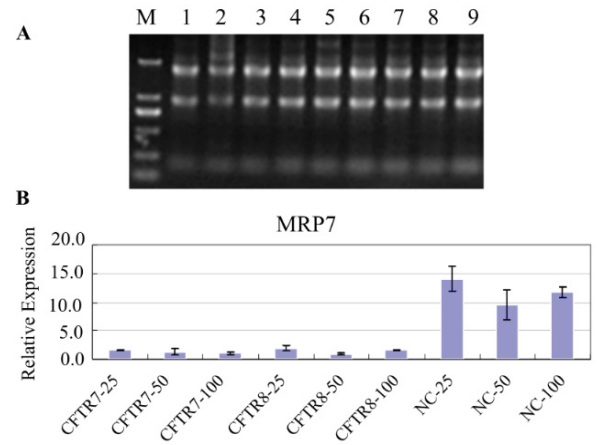

Figure 1: The mRNA expression of MRP7 **(A)** RNA electrophoregram. Lane 1: NC-25; Lane 2: NC-50; Lane 3: NC-100; Lane 4: GFTR7-NC-25; Lane 5: GFTR7-NC-50; Lane 6: GFTR7-NC-100; Lane 7: GFTR8-NC-25; Lane 8: GFTR8-NC-50; Lane 9: GFTR8-NC-100. M: Marker (the fragment size from the bottom to the top is 100, 250, 500, 750, 1000, and 2000 bp) and **(B)** The relative mRNA expression of MRP7 were detected by qRT-PCR after silencing MRP7 gene in HepG2 and SMMC7721 cells. Silencing MRP7 gene significantly decreased the mRNA level of MRP7. Quantification of mRNA expression was normalized by 18S RNA expression.

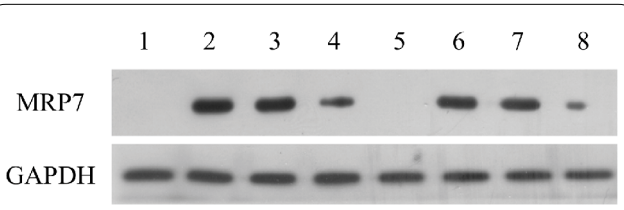

Figure 2: The protein level of MRP7. The protein level of MRP7 was measured by Western blot. Lane 1: HepG2; Lane 2:HepG2ADM; Lane 3: HepG2ADM-NC; Lane 4: HepG2ADM-MRP7siRNA; Lane 5: SMMC7721; Lane 6: SMMC7721ADM; Lane 7: SMMC7721ADM-NC; Lane 8: SMMC7721ADM-MRP7siRNA.

Table 1: Data analysis of western blot.

Sample	HepG2 Cell	HepG2 ADM	HepG2 NC	HepG2 Si MRP7	7721 Cell	7721 ADM	7721 NC	7721 Si MRP7
MRP7	36.75	2838.22	2501.26	925.35	3.62	1861.50	1620.36	249.29
GAPDH	1629.46	1644.07	1606.41	1642.35	1330.88	1413.09	1305.59	1103.36

Impact on invasive capacity of silencing MRP7 gene in SMMC7721/ADM and HepG2/ADM cells

In transwell invasion experiment, the average counts at high magnification (×100) of HepG2/ADM, HepG2/ADM-con, HepG2/ADM NCsiRNA and HepG2/ADM MRP7siRNA were 276,213,231,126. The results showed that the invasion ability of HepG2-ADM cells was significantly decreased after transfection with MRP7 siRNA (Figure 7).

The average counts of SMMC7721/ADM, SMMC7721/ADM-con, SMMC7721/ADM NCsiRNA and SMMC7721/ADM MRP7siRNA at high magnification (×100) were 136,139,145,76 respectively. The results showed that the invasion ability of SMMC7721-ADM cells was significantly decreased after transfection with MRP7 siRNA (Figure 8). Transwell assays revealed that silencing MRP7 gene significantly reduced the invasive potential of HepG2/ADM and SMMC7721/ADM cells (Table 4).

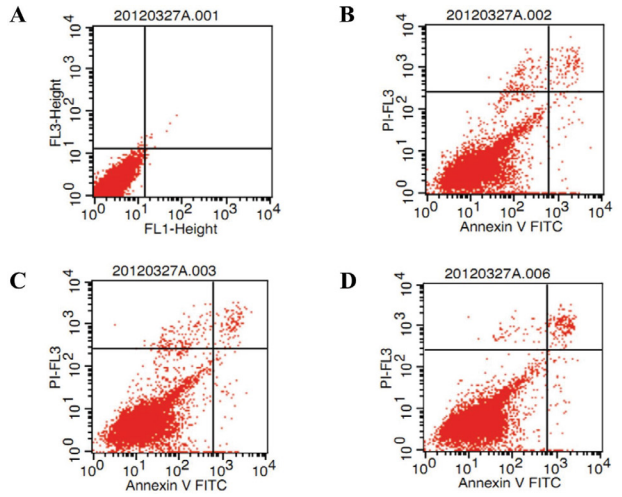

Figure 3: Results of HepG2ADM cell apoptosis. The apoptosis of HepG2ADM cells was analyzed by flow cytometry. **(A)** HepG2 ADM-Control, **(B)** HepG2 ADM, **(C)** HepG2 ADM NCsiRNA and **(D)** HepG2 ADM MRP7 siRNA.

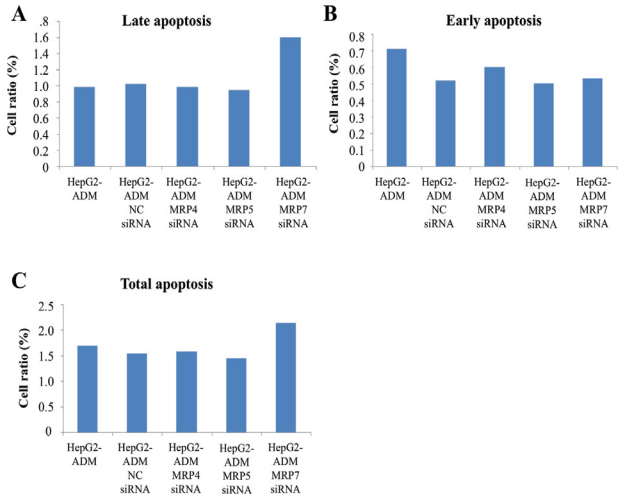

Figure 4: Statistics of HepG2ADM cell apoptosis. The apoptosis of HepG2ADM cells was analyzed by flow cytometry. **(A)** After silencing MRP7 gene, the proportion of late apoptotic cells was significantly increased, **(B)** After silencing MRP7 gene, the proportion of early apoptotic cells decreased slightly and **(C)** After silencing MRP7 gene, the total apoptosis of cells increased significantly.

Table 2: Data analysis of cell apoptosis.

Sample (%)	UL	UR	LL	LR	UR+LR
HepG2-ADM	1.14	0.98	97.17	0.71	1.69
HepG2-ADM NC siRNA	1.44	1.02	97.02	0.52	1.54
HepG2-ADM MRP7siRNA	0.53	1.60	97.34	0.53	2.13

Discussion

Primary hepatocellular carcinoma is one of the most common malignancies in the world today. In recent years, with the deepening understanding of liver cancer and related advances in science and technology, the treatment of liver cancer has made considerable development. In general, primary liver cancer has changed from an incurable disease to a partially curable disease. However, the development of the treatment of liver cancer is now facing a bottleneck stage. At present, the therapeutic effect of liver cancer is mainly attributed to the physical damage to the tumor, including surgical resection, freezing, radiofrequency and microwaves. The biggest

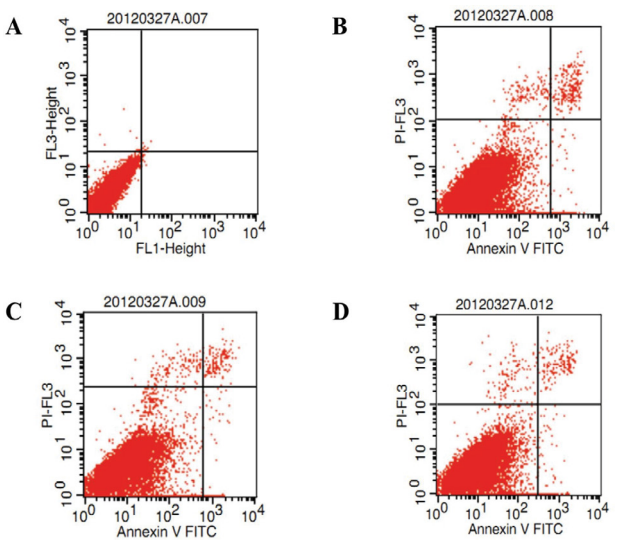

Figure 5: Results of SMMC7721/ADM cell apoptosis. The apoptosis of SMMC7721/ADM cells was analyzed by flow cytometry. **(A)** SMMC7721/ ADM-Control, **(B)** SMMC7721/ADM, **(C)** SMMC7721/ ADM NC siRNA and **(D)** SMMC7721/ADM MRP7 siRNA.

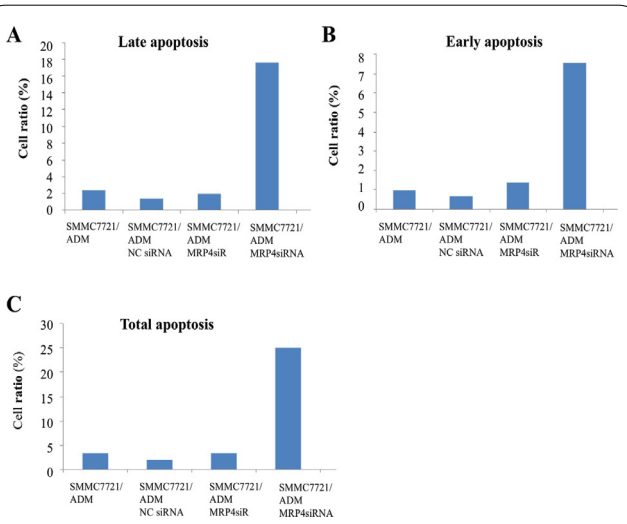

Figure 6: Statistics of SMMC7721/ADM cell late apoptosis. The apoptosis of SMMC7721/ADM cells was analyzed by flow cytometry. **(A)** After silencing MRP7 gene, the proportion of late apoptotic cells was significantly increased, **(B)** After silencing MRP7 gene, the proportion of early apoptotic cells decreased slightly and **(C)** After silencing MRP7 gene, the total apoptosis of cells increased significantly.

Table 3: Data analysis of cell apoptosis.

Sample (%)	UL	UR	LL	LR	UR+LR
SMMC7721-ADM	1.64	2.35	95.05	0.96	3.31
SMMC7721-ADM NC siRNA	1.31	1.32	96.72	0.65	1.97
SMMC7721-ADM MRP7siRNA	9.04	17.53	65.86	7.56	25.09

drawback of these treatments is that it is very difficult to completely kill the tumor, especially the tiny tumor lesions that are difficult to detect by the existing technical means [8,10]. Some other adjuvant treatments, including chemotherapy, immunotherapy and traditional Chinese medicine, have limited therapeutic effect on liver cancer.

Therefore, in order to achieve a breakthrough in the treatment of liver cancer, we must find ways to improve the efficacy of current adjuvant therapy and even make it be a major treatment. Immunotherapy is a promising development direction, but given the complex mechanisms of tumor immunity, to understand its mechanism may be just the tip of the iceberg so far and it is not realistic to make progress in the short term. Traditional Chinese medicine can only be an auxiliary treatment because of its own limitations. Chemotherapy has been used for a long time in the treatment of cancer with affirmative curative effect and it has achieved excellent results in the treatment of some tumors and it can cure some malignant tumors, for example, some types of leukemia and some types of choriocarcinoma [15,16]. Therefore, it has more practical significance to find ways in the chemotherapy of liver cancer.

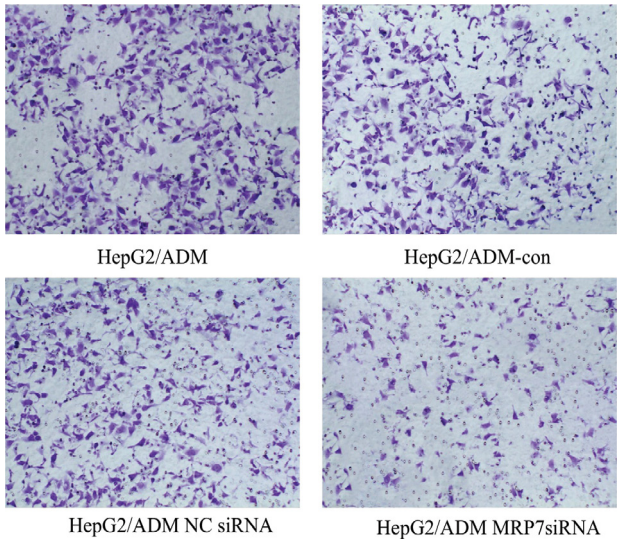

Figure 7: The invasion capacity of HepG2/ADM cells. The invasion capacity of cells in the HepG2/ADM, HepG2/ADM-control, HepG2/ADM NC siRNA, and HepG2/ADM MRP7 siRNA groups was evaluated by Transwell assay.

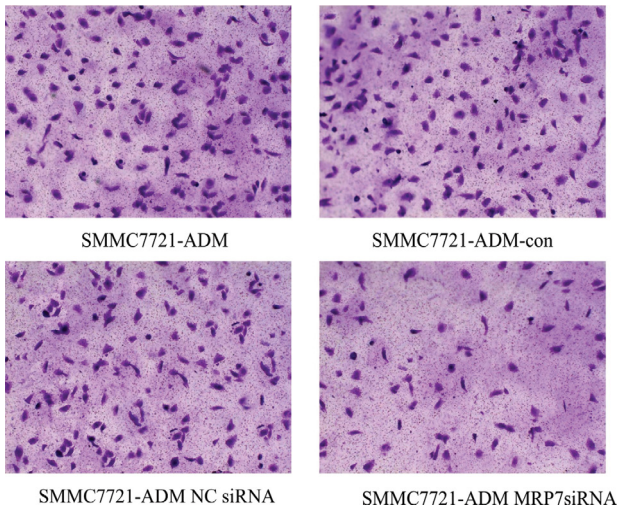

Figure 8: The invasion capacity of SMMC7721/ADM cells. The invasion capacity of cells in the SMMC7721/ADM, SMMC7721/ADM-control, SMMC7721/ADM NC siRNA, and SMMC7721/ADM MRP7siRNA groups was evaluated by Transwell assay.

Table 4: The average cell counts in transwell invasion experiment.

Sample	Blank	Control	NC siRNA	MRP7siRNA
HepG2/ADM	276	213	231	126
SMMC7721/ADM	136	139	145	76

SiRNA is a relatively mature gene transcription technology at the transcriptional level, which can specifically and efficiently block gene expression and therefore used as a simple and effective gene knockout tool [17-19]. Its action mechanism is as follows.

Double-stranded RNA within the biological cells is identified cleaved by Dicer to generate 21-23nt siRNA. The complex of this siRNA and a series of nucleases is called the RNA induced silencing complex (RISC), which includes helicases and endonucleases. The helicases transform the siRNA double chain into the activated form which is complementary to the target sequence mRNA and is called the antisense siRNA. The antisense siRNA mediated the identification of the activated complex and combined with the specific complementarity mRNA, and ultimately guided nucleic acid endonuclease to cut the mRNA of target sequence into pieces to make sense [20]. Nisana, et al. [21] found that the IC50 of chemotherapy drugs of hilar cholangiocarcinoma such as doxorubicin and pirarubicin was highly related with MRP7expression, which suggested that MRP7 expression may affect the resistance of some chemotherapy drugs. In this study, the corresponding siRNA targeted MDR7 gene for liver cancer cell was designed and then transfected into HepG2/ADM and SMMC7721/ADM resistant cells to produce siRNA to make sense.

In our study, human hepatocellular carcinoma cell lines HepG2 and SMMC-7721 were used as parental cells and SMMC-7721/ADM and HepG2/ADM cell lines were established according to the same induction method and its drug resistance was identified. MRP7 mRNA expression was detected by qRT-PCR after silencing MRP7 gene in HepG2 and SMMC7721 cells. Silencing MRP7 gene significantly decreased MRP7 mRNA expression levels (Figure 1). Western blot results showed that MRP7 protein was expressed in HepG2 cells (control group), HepG2/ADM cells (resistant group), HepG2/ADM transfected MRP7-siRNA group (interference group) andSMMC7721 cells (control group), SMMC7721/ADM cells (resistant group) and SMMC7721/ADM transfected MRP7-siRNA group (interference group).The expression of MRP7 protein in the interference group was significantly lower than that in the drug-resistant group and the control group (Figure 2 and Table 1). Detect cell apoptosis before and after MRP7-siRNA transfection by flow cytometry and we found that silencing MRP7 gene resulted in a significantly higher rate of apoptosis of HepG2/ADM and SMMC7721/ADM cells. MRP7 gene was highly related with cell apoptosis (Figures 3-6, Tables 2 and 3). In transwell invasion experiment, the average counts at high magnification (X 100) of HepG2-ADM, HepG2-ADM-con, HepG2- ADM NCsiRNA and HepG2-ADM MRP7siRNA were 276,213,231,126. The results showed that the invasion ability of HepG2-ADM cells was significantly decreased after transfection with MRP7 siRNA. The average counts of SMMC7721-ADM, SMMC7721-ADM-con, SMMC7721-ADM NCsiRNA and SMMC7721-ADM MRP7siRNA were 136,139,145,76 respectively. The results showed that the invasion ability of SMMC7721-ADM cells was significantly decreased after transfection with MRP7 siRNA. Transwell assays revealed that silencing MRP7 gene significantly

reduced the invasive potential of HepG2/ADM and SMMC7721/ADM cells (Figures 7 and 8).

Conclusion

In conclusion, the silencing of MRP7 gene by siRNA silencing drug-resistant HepG2/ADM and SMMC-7721/ADM cells significantly increased the sensitivity of chemotherapeutic drugs and even reversed the drug resistance of chemotherapeutic drugs. MRP7 plays an important role in the occurrence and development of multidrug resistance in hepatocellular carcinoma and causes primary or secondary drug resistance of hepatoma cells to chemotherapeutic drugs. Therefore, it is necessary in clinical practice to detect MRP7 in chemo resistant patients with hepatocellular carcinoma to evaluate the efficacy of chemotherapy.

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Ethics Approval and Consent to Participate

The ethic approval was obtained from the Ethic Committee of Tongji Medical College, Huazhong University of Science and Technology.

Consent to Publish

All of the authors have consented to publish this research.

Availability of Data and Materials

The data are free access to available upon request.

Competing Interests

All authors declare no conflict of interest.

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Authors' Contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

References

1. Bruix, J., Han, K. H., Gores, G., Llovet, J. M., & Mazzaferro, V.
   Liver cancer: Approaching a personalized care.
   J Hepatol., 2015;**62**(1 Suppl): S144-S156.
2. Kudo, M.
   Chronic liver diseases and liver cancer: An update in 2015.
   Dig Dis., 2015;**33**(6): 705-707.
3. Wei, K. R., Yu, X., Zheng, R. S., Peng, X. B., Zhang, S. W., Ji, M. F., et al.
   Incidence and mortality of liver cancer in China, 2010.
   Chin J Cancer., 2014;**33**(8): 388-394.
4. Fan, J. H., Wang, J. B., Jiang, Y., Xiang, W., Liang, H., Wei, W. Q., et al.
   Attributable causes of liver cancer mortality and incidence in China.
   Asian Pac J Cancer Prev., 2013;**14**(12): 7251-7256.
5. Marengo, A., Rosso, C., & Bugianesi, E.
   Liver cancer: connections with obesity, fatty liver, and cirrhosis.
   Annu Rev Med., 2016;**67**: 103-117.
6. Yang, Y., Gao, J., Li, H. L., Zheng, W., Yang, G., Zhang, W., et al.
   Dose-response association between hepatitis B surface antigen levels and liver cancer risk in Chinese men and women.
   Int J Cancer., 2016;**139**(2): 355-362.
7. Guglielmi, A., Ruzzenente, A., Conci, S., Valdegamberi, A., Vitali, M., Bertuzzo, F., et al.
   Hepatocellular carcinoma: surgical perspectives beyond the barcelona clinic liver cancer recommendations.
   World J Gastroenterol., 2014;**20**(24): 7525-7533.
8. Sun, T. Y., Yan, W., Yang, C. M., Zhang, L. F., Tang, H. L., Chen, Y., et al.
   Clinical research on dendritic cell vaccines to prevent postoperative recurrence and metastasis of liver cancer.
   Genet Mol Res., 2015;**14**(4): 16222-16232.
9. Li, S., & Zheng, L.
   Effect of combined treatment using wilfortrine and paclitaxel in liver cancer and related mechanism.
   Med Sci Monit., 2016;**22**: 1109-1114.
10. Fortner, J. G., Mulcare, R. J., Solis, A., Watson, R. C., & Golbey, R. B.
    Treatment of primary and secondary liver cancer by hepatic artery ligation and infusion chemotherapy.
    Ann Surg., 1973;**178**(2): 162-172.
11. Wang, C. F., Wang, Y. Q., Huang, F. Z., Nie, W. P., Liu, X. Y., & Jiang, X. Z.
    Association between reversal of multidrug resistance by methyl jasmonate and P-glycoprotein ATPase activity in hepatocellular carcinoma.
    J Int Med Res., 2013;**41**(4): 964-974.
12. Slot, A. J., Molinski, S. V., Cole, & S. P. C.
    Mammalian multidrug resistance proteins (MRPs).
    Essays Biochem., 2011;**50**(1): 179-207.
13. Korita, P. V., Wakai, T., Shirai, Y., Matsuda, Y., Sakata, J., Takamura, M., et al.
    Multidrug resistance-associated protein 2 determines the efficacy of cisplatin in patients with hepatocellular carcinoma.
    Oncology Rep., 2010;**23**(4): 965-972.
14. Folmer, Y., Schneider, M., Blum, H. E., & Hafkemeyer, P.
    Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-ABCC2 antisense constructs.
    Cancer Gene Ther., 2007;**14**(11): 875-884.
15. Seo, S., Hatano, E., Higashi, T., Nakajima, A., Nakamoto, Y., Tada, M., et al.
    P-glycoprotein expression affects 18F-fluorodeoxyglucose accumulation in hepatocellular carcinoma *in vivo* and *in vitro*.
    Int J Oncol., 2009;**34**(5): 1303-1312.
16. Zhao, J., Yu, B. Y., Wang, D. Y., & Yang, J. E.
    Promoter polymorphism of MRP1 associated with reduced survival in hepatocellular carcinoma.
    World J Gastroenterol., 2010;**16**(48): 6104-6110.
17. Ye, L., Zhao, X., Lu, J., Qian, G., Zheng, J. C., & Ge, S.
    Knockdown of TIGAR by RNA interference induces apoptosis and autophagy in HepG2 hepatocellular carcinoma cells.
    Biochem Biophys Res Commun., 2013;**437**(2): 300-306.
18. Zeng, X. C., Zhang, T., Huang, D. H., Wang, G. Y., Chen, W., Li, H., et al.
    RNA interfering targeting human leukocyte antigen-G enhanced immune surveillance mediated by the natural killer cells on hepatocellular carcinoma.
    Ann Clin Lab Sci., 2013;**43**(2): 135-144.
19. Nisana, T., Liengchai, C., Banchob, S., Namwat, W., Wongkham, S., Bhudhisawasdi, V., et al.
    Drug sensitivity and drug resistance profiles of human intrahepatic cholangiocarcinoma cell lines.
    World J Gastroenterol., 2005;**11**(18): 2748-2753.
20. Mai, L., Yang, L., Kuang, J. Y., Zhang, S. Q., Kang, Y. H., Xu, Q. H., et al.
    Small interfering RNA targeting to hepatitis B virus X gene and 5-aza-2 -deoxycytidineon inhibited growth of the subcutaneous implanted tumor of hepatocellular carcinoma in nude mice.
    Zhong Hua Shi Yan He Lin Chuang Bing XueZaZhi., 2012;**26**(5): 362-365.
21. Namwat, N., Amimanan, P., Loilome, W., Jearanaikoon, P., Sripa, B., Bhudhisawasdi, V., et al.
    Characterization of 5-fluorouracil- resistant cholangiocarcinoma cell lines.
    Chemotherapy., 2008;**54**(5): 343-351.