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Therapeutic Effect of Down-Regulation of MTH1 Gene Expression on HepG2 Hepatocellular Carcinoma in Nude Mice

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Abstract: The aim of this study was to establish an animal model of HepG2 liver cancer in nude mice, and to inhibit the expression of MTH1 gene in tumor cells by siRNA interference. To study the therapeutic effect of down-regulation of MTH1 expression on HepG2 tumor in nude mice, and to provide a new strategy for the treatment of HCC. Methods: Human HepG2 cells were cultured and transplanted tumor models were established under the armpit of right forelimb of nude mice. The successful nude mice were randomly divided into two groups: control group (CT) and siRNA transfection group (MTH1). The tumor volume of each group of nude mice was consistent. This experiment was mainly conducted by injecting siRNAs into tumor-bearing nude mice and transfection reagents in vivo. The survival time and living condition of nude mice were observed. The tumor inhibition was evaluated by measuring the volume and weight of solid tumor in nude mice. After the administration, The expression of mth1 and its related genes were detected by qPCR to determine the effect of QPCR on the mRNA expression of each gene. The relative expression of mth1 and related proteins in tumor tissues was detected by Elisa and Western blotting. The effect of siRNA on the protein level of each gene was evaluated by calculating the gray value and comparing the od value. Results: The animal model of HepG2 liver cancer in nude mice was successfully established, and the tumorigenesis rate was 100%. The siRNA transfection group had significant anti-tumor effect and significantly inhibited the growth of tumor cells. Survival rate of nude mice in each group was 100%. Compared with the control group, In the transfected group, the expression of mth1 mRNA and protein decreased significantly in the tumor tissues. The expression of caspase-3 protein was significantly increased in the transfected group. Conclusion: SiRNA-MTH1 can effectively silence the expression of MTH1 gene. Down-regulation of mth1 gene expression by siRNA can inhibit the growth, proliferation and apoptosis of HepG2 tumor cells.

Keywords: Nude mice, HepG2 CELL, saRNA, MTH1

Introduction

Primary liver cancer (HCC) is one of the most common malignant tumors in the world, its morbidity and mortality are among the highest in the world[1]. The liver cancer has the characteristics of concealment, long latency, rapid development, high malignancy and so on. Surgical treatment is the best method for the treatment of liver cancer[2]. However, because most patients were diagnosed only in the late stages of the disease, they missed the best time for surgical treatment. Therefore, the search for new therapeutic tools and drug targets is essential for the treatment of liver cancer[3].

MTH1 (mutT homolog1) is a homologous enzyme of MutT, which is widely distributed in the nucleus and mitochondria and participates in DNA damage and repair. especially in the repair of DNA damage of tumor cells. It is shown that MTH1 can scavenge the oxidized dNTPs, prevent them from participating in DNA replication and prevent DNA damage[4]. MTH1 is highly expressed in a variety of tumor cells, but not or rarely expressed in healthy tissue cells. MTH1 is considered to be an important repair gene in tumor cells[5,6].

The occurrence and development of liver cancer is a complex multistage process. It is the long-term accumulation of multiple gene variants and is closely related to the increase of proto oncogene expression. Therefore, targeted therapy for proto-oncogene is more effective. The purpose of this study was to investigate the effects of simultaneous targeting proto-oncogene therapy on the biological behavior of HepG2 cells. We

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designed and synthesized siRNA sequences targeting MTH1 gene and transfected them into HepG2 cells in vitro. To investigate the effect of down-regulation of MTH1 gene expression on the proliferation, invasion and apoptosis of HepG2 cells. To provide a theoretical basis for gene targeting therapy of cancer.

MATERIALS AND METHODS

Cell Culyures

HepG2 cells were grown in Roswell Park Memorial Institute medium (RPMI-1640) (Hyclone, GE Healthcare Life Sciences, Logan, UT, USA), containing 10% fetal bovine serum (Gibco™; Thermo Fisher Scientific, Inc, Carlsbad, CA, USA), 100U/ml penicillin and 100 μg/ml strepto-mycin, and incubated at 37˚C in a humidified atmosphere of 5% CO_2. Cells in exponential growth phase were used throughout the experiments.

Establishment of Nude Mouse Model

After 6 days of inoculation, the tumor grows to a certain size. According to the size of tumor, they were divided into control group and siRNA-MTH1 transfection group. There were 6 mice in each group, and the tumor volume of each group was basically the same. The control group was given transfection reagent, SiRNA-MTH1 transfection group was given, each of them was 19.8 ug. Once every 3 days next to the tumor, lasting one month.

Observation on living condition of tumor-bearing nude mice

The activity, tumor growth, food intake and water intake were observed daily and the living conditions of the mice were recorded. The long and short diameters of the tumor were measured every 5 days, and the volume size of the tumor was calculated and recorded. $V= 1 / 2 \times ab^2$(a is long diameter and B is short diameter). Tumor growth inhibition rate (control group tumor weight-experimental group tumor weight / control group tumor weight × 100%).

Detection of relative mRNA expression in tumor tissues

Real-time fluorescence quantitative PCR: To remove the total RNA by using Trizol reagent by conventional method. Total gold reverse transcription kit was used to reverse the RNA into cDNA. then the cDNA was amplified by QPCR. β-actin gene primers: The upstream sequence is 5'-ATGGGTCAGAAGGACTCCTATG-3', The downstream sequence is 5 '-ATCTCCTGCTCGAAGTCTAGAG-3'. Mth1 gene primers: Upstream primer sequence 5'-GTGCAGAACCCAGGGACCAT-3', Sequence of downstream primers 5'-GCCCACGAACTCAAACACGA-3'. QPCR reaction conditions: Predenaturation at 94 ℃ for 45 s, denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 30 s, extending at 72 ℃ for 30 s, cycling 40 times, and extending at 72 ℃ for 5 min. PCR results were analyzed by Graphpad Primer6.0 software.

Determination of protein expression in tissues

The relative expression of MTH1 protein was detected by enzyme-linked immunosorbent assay (Elisa). The relative expression of apoptotic protein caspase-3 was detected by Western blot assay. The tumor tissue of 100mg was removed, and the blood stain was washed by PBS. The tissue was lapped in 1ml RIPA for the night and then centrifuged to extract the supernatant. The tissue protein solution. Then according to the instructions of Elisa kit and the method of Western imprinting.

Statistical treatment

SPSS 17.0 statistical software was used to analyze the data. The experimental data are expressed as mean ±standard deviation, single factor analysis of variance was used for statistical analysis. $P < 0.05$ The difference is statistically significant.

RESULTS

Observation on general physiological conditions of tumor-bearing nude mice

HepG2 cells were all inoculated 7 days later, and the tumor formation rate was 100%. The survival condition of the mice was good, and there was no significant difference between the groups. With the development of the experiment, the tumor growth rate of siRNA transfected mice was slower than that of control group. In the later stage of experiment, the control group gradually suffered from loss of appetite and abnormal activity.

Effect of siRNA administration on the volume and quality of nude tumor

30 days after administration, the tumor size, volume, weight and growth rate of the two groups were compared. The tumor growth rate of nude mice in transfection group was significantly lower than that in control group (Fig.1A). The tumor size in the transfection group was significantly smaller than that in the control group (Fig.1B). The tumor weight in the transfection group was significantly lower than that in the control group (fig. 1C). There were significant differences in volume and tumor weight between the transfection group and the transfection group. There was statistical significance ($P < 0.05$). The result is as follows.

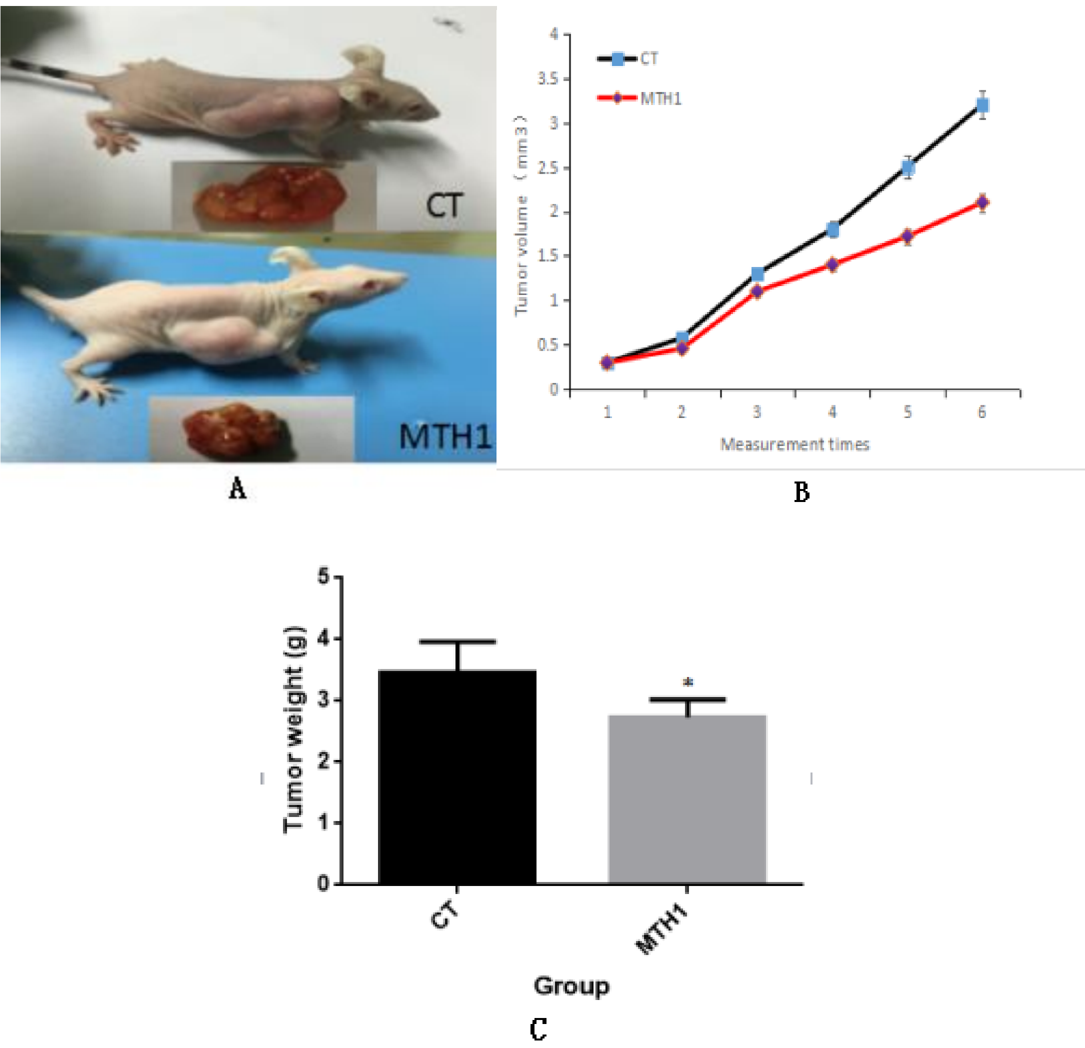

Fig. 1A The tumor size in the transfection group was significantly smaller than that in the control group.
Fig. 1B The tumor growth rate in the transfection group was significantly lower than that in the control group.
Fig. 1C The tumor weight of the transfected group was significantly lower than that of the control group.
These results indicated that siRNA-MTH1 could significantly inhibit the growth of HepG2 hepatoma cells.

Determination of MTH1 mRNA relative expression in tumor tissues

The effect of siRNA on tumor mRNA was detected by qPCR. The results of qPCR showed that (fig. 2) the expression of MTH1 mRNA in tumor tissue of siRNA-MTH1 transfection group was significantly lower than that of control group ($P < 0.05$). The results showed that siRNA-MTH1 could inhibit the expression of proto-oncogene mth1.

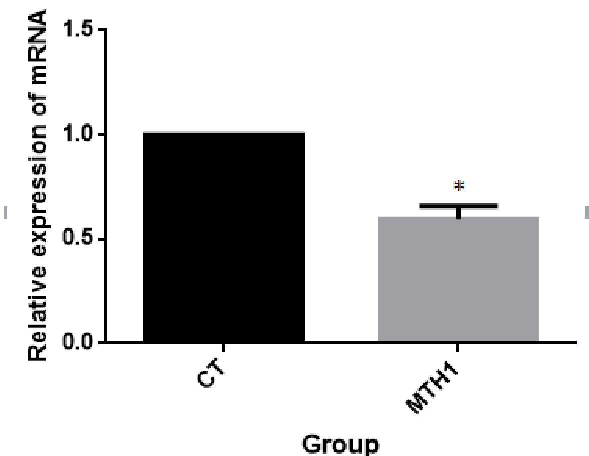

Fig. 2 The mRNA expression of MTH1 gene in tumor tissue in transfected group was significantly lower than that in control group, and the difference was statistically significant(*: P <0.05).

Detection of MTH1 and caspase-3 protein relative expression in tumor tissues

The expression of mth1 protein in the transfected group was significantly lower than that in the control group (Fig. 3A). The expression of caspase-3 protein in the transfected group was significantly higher than that in the control group (*: P <0.05). The relative expression level was detected by ELISA in tumor tissue. The expression of mth1 protein in the transfected group was significantly lower than that in the control group(**: P <0.01)(Fig. 3B).

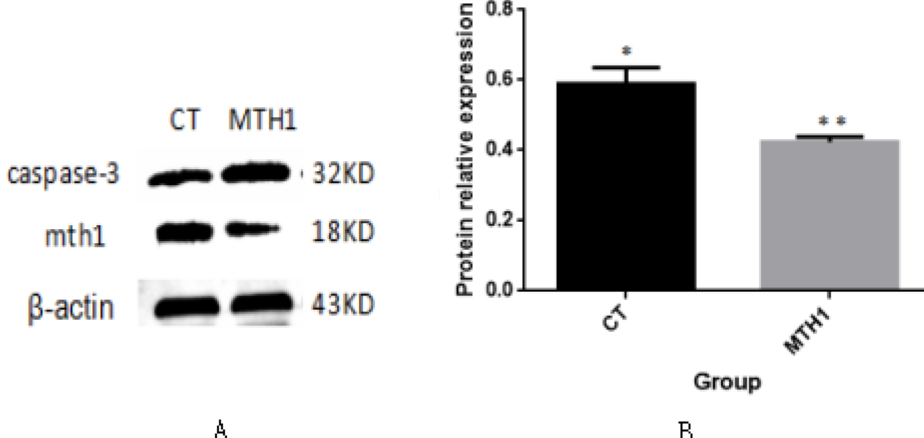

Fig. 3A Western blotting was used to detect the relative expression of MTH1 and caspase-3 protein in tumor tissues. The siRNA transfection group was compared with the control group, the expression of mth1 protein decreased significantly and the expression of caspase-3 protein increased significantly.

Fig. 3B Elisa method was used to detect the relative expression of mth1 protein in tumor tissues. The expression of mth1 protein in the transfected group was significantly lower than that in the control group.

DISCUSSION

The best-known small, non-coding RNA that suppresses gene expression falls into three broad categories: Small RNAs miRNAs, small interference RNAs siRNAs, and interactions with PIWI protein RNAs piRNAs[7,8,9]. The mature forms of these RNAs are about 20-30 nucleotide nts, which are associated with many ago protein superfamily members and play a key role in RNA interference RNAi pathway[10-12]. It is generally believed that miRNA and siRNA mediate post-transcriptional gene silencing, guide the complement of ago and mRNA in cytoplasm to form silencing complex, induce transcriptional degradation and prevent post-transcriptional translation[8,11]. PIWI, a branch of the PiRNA and ago protein family, binds to the silent germ cell transposon and plays a role in many biological fertility[13,14].

Although many preliminary studies have focused on the mechanism of gene silencing mediated by small and medium-sized RNAs in the cytoplasm, it has also been shown that many of these RNAs can also mediate epigenetic transcriptional silencing in the nucleus[15-19]. It is proved that ago protein is a key factor in the mechanism of nuclear RNAi, as well as its importance and versatility as a gene silencing complex. RNA-induced transcriptional silencing complex, mainly loaded with a small ribonucleic acid, can mediate cotranscriptional gene silencing[20,21].

In this study, mth1 gene expression in tumor cells was silenced by RNAi technique, and the therapeutic effect of down-regulation of MTH1 expression on nude mice HepG2 hepatoma tumor was studied. The results showed that siRNA-MTH1 could silence the expression of MTH1 gene, and down-regulate the proliferation and invasion of HepG2 tumor after the expression of MTH1 gene was down-regulated. The ability of apoptosis was enhanced. In general, down-regulation of MTH 1 gene expression significantly inhibited the growth of tumor cells. At present, the majority of targeted immunotherapeutic drugs are antibody drugs and small molecular drugs. Small molecular drugs belong to chemical drugs, which have toxic side effects on the body, also cause damage to normal cells, and may also cause harm to people in the process of production. The antibody is a protein, high cost and long-term use of the body will cause immune response, but also produce side effects on the body. The purpose of this study is to find a substitute or method to reduce the side effects on the body and to effectively inhibit the proliferation of tumor cells at the same time. SiRNA is easy to produce and low in cost. It does not produce immune tolerance and is a biomolecules. It is metabolized quickly in vivo, so it has no side effects. This study is an attempt to search for siRNA anti-cancer drugs. The combination of siRNA and siRNA may enhance the anticancer effect, make it synergistic, and kill tumor cells to the maximum extent. Therefore, the use of RNAi targeted immunotherapy may become a hot topic in tumor therapy and an important part of accurate therapy.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012[J]. International Journal of Cancer, 2015, 136(5): E359.
2. Jemal A. Global Cancer Statistics (vol 61, pg 69, 2011)[J]. Ca A Cancer Journal for Clinicians, 2011, 61(2): 134-134.
3. Mikhail S, Cosgrove D, Zeidan A. Hepatocellular carcinoma: systemic therapies and future perspectives[J]. Expert Review of Anticancer Therapy, 2014, 14(10): 1205-1218.
4. Asal F, Winta Woldai H, Marina I, et al. Analysis of mutant frequencies and mutation spectra in hMTH1 knockdown TK6 cells exposed to UV radiation[J]. Mutation Research/fundamental & Molecular Mechanisms of Mutagenesis, 2013, 751-752(5):8.
5. Gad H, Koolmeister T, Jemth A S, et al. MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool[J]. Nature, 2014, 508(7495):215-21.
6. Helleday T. Cancer phenotypic lethality, exemplified by the non-essential MTH1 enzyme being required for cancer survival[J]. Annals of Oncology, 2014, 25(7):1253.
7. Castel S E, Martienssen R A. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond[J]. Nature Reviews Genetics, 2013, 14(2): 100-112.
8. Carthew R W, Sontheimer E J. Origins and mechanisms of miRNAs and siRNAs[J]. Cell, 2009, 136(4): 642-655.
9. Thomson T, Lin H. The biogenesis and function PIWI proteins and piRNAs: progress and prospect[J]. Annual review of cell and developmental biology, 2009, 25: 355.
10. Kim V N, Han J, Siomi M C. Biogenesis of small RNAs in animals[J]. Nature reviews Molecular cell biology, 2009, 10(2): 126-139.
11. Bartel D P. MicroRNAs: genomics, biogenesis, mechanism, and function[J]. cell, 2004, 116(2): 281-297.
12. Song J J, Liu J, Tolia N H, et al. The crystal structure of the Argonaute2 PAZ domain reveals an RNA binding motif in RNAi effector complexes[J]. Nature Structural & Molecular Biology, 2003, 10(12): 1026-1032.
13. Bagijn M P, Goldstein L D, Sapetschnig A, et al. Function, targets, and evolution of Caenorhabditis elegans piRNAs[J]. Science, 2012, 337(6094): 574-578.
14. Landry C D, Kandel E R, Rajasethupathy P. New mechanisms in memory storage: piRNAs and epigenetics[J]. Trends in neurosciences, 2013, 36(9): 535-542.
15. Volpe T A, Kidner C, Hall I M, et al. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi[J]. science, 2002, 297(5588): 1833-1837.
16. Robb G B, Brown K M, Khurana J, et al. Specific and potent RNAi in the nucleus of human cells[J]. Nature structural & molecular biology, 2005, 12(2): 133-137.
17. Burton N O, Burkhart K B, Kennedy S. Nuclear RNAi maintains heritable gene silencing in Caenorhabditis elegans[J]. Proceedings of the National Academy of Sciences, 2011, 108(49): 19683-19688.
18. Morris K V, Chan S W L, Jacobsen S E, et al. Small interfering RNA-induced transcriptional gene silencing in human cells[J]. Science, 2004, 305(5688): 1289-1292.
19. Weinberg M S, Villeneuve L M, Ehsani A L I, et al. The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells[J]. Rna, 2006, 12(2): 256-262.
20. Bühler M, Verdel A, Moazed D. Tethering RITS to a nascent transcript initiates RNAi-and heterochromatin-dependent gene silencing[J]. Cell, 2006, 125(5): 873-886.
21. Guang S, Bochner A F, Burkhart K B, et al. Small regulatory RNAs inhibit RNA polymerase II during the elongation phase of transcription[J]. Nature, 2010, 465(7301): 1097-1101.