Original Article

MicroRNA-497 induces apoptosis through downregulating XIAP in hepatic cancer

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Abstract: Hepatic cancer is one of the important malignant tumors in digestive system. MicroRNAs regulate cell growth and other cell behaviors through signaling pathway. This study investigated the role of microRNA-497 on LM3 cell viability, proliferation, and apoptosis. MicroRNA-497 and scramble microRNA (miRNA) were synthetized and transfected to hepatic cancer cell line LM3 using lipofectamine. LM3 cell growth, proliferation, and apoptosis were evaluated by MTT assay, caspase-3 activity detection, and flow cytometry. XIAP siRNA or plasmid was synthetized and transfected to LM3 cells before microRNA-497 transfection. XIAP protein expression was detected by Western blot. MicroRNA-497 transfection obviously reduced LM3 cell proliferation (P = 0.0068), increased phosphatidylserine eversion (P = 0.017), enhanced caspase-3 activation (P = 0.023), and downregulated XIAP expression level (P = 0.024). XIAP interference enhanced the effect of microRNA-497 on inducing LM3 cell apoptosis (P = 0.0067), while XIAP overexpression inhibited LM3 cell apoptosis triggered by microRNA-497 (P = 0.0082). MicroRNA-497 suppressed hepatic cancer cell line LM3 cell proliferation and induced cell apoptosis through downregulating XIAP, suggesting that XIAP might be a potential treatment target for hepatic cancer.

Keywords: microRNA-497, XIAP, LM3 cell, cell apoptosis

Introduction

Hepatic cancer is an important malignant tumor in digestive system [1, 2]. At present, comprehensive treatment is usually applied in hepatic cancer therapy [3, 4]. Though the curative effect of combined therapy is significant in clinic, it still exhibits various shortcomings and deficiencies, such as bleeding and other side effects [5-7]. How to improve the accuracy and success rate of hepatic cancer treatment is important and difficult in medical and scientific community.

Molecular targeted therapy is the development trend of hepatic cancer [8-10]. However, the effect of current molecular targeted treatment on hepatic cancer is still poor [11]. Therefore, it is urgently needed to find more effective molecular targets for hepatic cancer in clinic [11, 12]. Moreover, it is still lack of the therapy targeting microRNAs in hepatic cancer treatment [12].

MicroRNAs have multiple functions on cell, including regulating cell growth and signaling pathway. For instance, microRNA-218 can inhibit hepatic cancer cell growth, whereas microRNA-34a is associated with tumor metastasis [13, 14]. It suggested that microRNAs may also participate in the occurrence and development of hepatic cancer [13-15]. MicroRNA-497 is found to be obviously upregulated in hepatic cancer tissue compared with the corresponding adjacent tissue, indicating that microRNA-497 may be involved in hepatic cancer progress [16, 17]. This study intended to explore the possible regulatory function of microRNA-497 on hepatic cancer cell line LM3.

The anti-tumor strategy is to kill tumor cells without affecting normal cells. Cell apoptosis is regulated by anti-apoptotic proteins and pro-apoptotic proteins [18, 19]. The ideal anti-cancer drugs can decline anti-apoptotic protein expression and upregulate pro-apoptotic protein level in cancer cells. XIAP protein is a kind of widely studied anti-apoptotic molecule [20, 21]. Currently, although there are many drugs targeting XIAP protein, their effect in regulating XIAP protein level is still unsatisfactory. Further-
microRNA-497 regulates XIAP in hepatic cancer

more, they may cause bleeding and other side effects [22]. This study also attempted to explore the potential molecules that can target XIAP protein.

Therefore, this study intended to investigate the role of microRNA-497 on hepatic cancer cell line LM3 and related mechanism, aiming to provide the theoretical basis for hepatic cancer treatment target selection.

Materials and methods

Reagents

XIAP and actin antibodies were from Santa Cruz (CA, USA). Fetal bovine serum and cell culture medium were from Beijing Hualan Bioengineering Company (Beijing, China). MTT was got from Beijing Dingguo Changsheng Bio-
technology co., Ltd (Beijing, China). Apoptosis detection reagent FITC-annexin and caspase 3 detection kit were bought from Beyotime (Jiangsu, China).

MicroRNA-497 (5’-AATCGGTA-GTCATCTGCCC-3’ and 5’-AAAGTAAAGACAAAGGTGCA-3’), scramble microRNA (5’-CCTGTCTTTCCAAGGAGAC-3’ and 5’-TTGTCAGTACGTTGCA-AT-3’), XIAP siRNA (5’-CTAACCATGGCGTGCTATGC-3’ and 5’-TTATATCACTAGCTAGTTG-3’), and XIAP plasmid were purchased from GenePharma. Lipofectamine transfection kit was bought from Invitrogen.

Cell culture

Hepatic cancer cell line LM3 cells were resuscitated and suspended in high glucose DMEM medium. The cells were cultured at 37°C and 5% CO2 [9].

Transfection

MicroRNA-497 and microRNA were transfected to LM3 cells by conventional lipofectamine method. Specially, LM3 cells were seeded in 24-well plate at the density of 68%. A total of 0.5 μl microRNA-497 or microRNA (1 μg/μl) were mixed in lipofectamine 2000 and added to the cells [9]. The transfection efficiency was detected to be higher than 95%.

MTT assay

LM3 cells viability was determined by conventional MTT assay [10]. The cells were seeded in 96-well plate and incubated for 8 h. After transfected by microRNA-497 or microRNA, the cells were treated by MTT at 1 mg/ml for 5 h. Next, the plate was added with DMSO to stop the reaction and tested on microplate reader at 420 nm to obtain the absorbance value [11].

Cell apoptosis detection

LM3 cell apoptosis was stained by Annexin-V-FITC and tested by flow cytometry. LM3 cells were transfected by microRNA-497 or microRNA for 48 h. Then the cells were collected and added with Annexin-V-FITC for 15 min. At last, the cells were tested on flow cytometry to evaluate cell apoptosis [14].
microRNA-497 regulates XIAP in hepatic cancer

Western blot

Total protein was extracted from LM3 cells and quantified using BCA method. A total of 15 μg protein was boiled and separated by SDS-PAGE. After transferred to PVDF membrane, the protein was blocked for 1.5 h. Next, the membrane was incubated in primary antibody (1:800) at 4°C overnight and then incubated in secondary antibody (1:1500) at 37°C for 2 h. At last, the membrane was washed by TBST and treated by ECL reagent for development. Gel imaging system from Shanghai Qinxiang scientific instrument co., Ltd was used to analyze XIAP protein expression in LM3 cells [15].

Caspase-3 activity detection

Caspase-3 activity in LM3 cells was determined by detection kit. LM3 cells were transfected by microRNA-497 or microRNA and resuspended in DMEM medium. Then the cells were incubated in chromophoric substrate at room temperature and tested on microplate reader [17]. Relative caspase-3 activity was calculated as follows: (Ab in microRNA-497 group - Ab in microRNA group)/Ab in microRNA group.

XIAP transfection

To test XIAP impact on LM3 behavior after microRNA-497 transfection, LM3 cells were first transfected with XIAP siRNA or plasmid, followed by microRNA-497 or microRNA transfection.

LM3 cells were seeded at the density of 65% and then added with lipofectamine 2000 solution containing 2 μl XIAP siRNA or XIAP plasmid (1.6 μg/μl). Next, the cells were treated by 2 μl microRNA-497 or microRNA (0.6 μg/μl) transfection for further analysis.

Data analysis

All data were analyzed by SPSS 13.0 software. The measurement data was compared by t test. A P < 0.05 was considered as statistical significance.

Results

MicroRNA-497 transfection declined LM3 cell viability

Hepatic cancer LM3 cell viability was detected by MTT assay. It was showed that compared with LM3 cells transfected by 0.5 μg miRNA, LM3 cells transfected by 0.5 μg microRNA-497 exhibited obviously lower cell viability (P = 0.0068) (Figure 1).

Since no statistical difference was observed on cell viability between LM3 transfected or untransfected by miRNA (P > 0.05), we used LM3 transfected by miRNA as control in the following investigation.

MicroRNA-497 induced LM3 cell apoptosis

Annexin-V-FITC was applied to detect LM3 cell apoptosis. It was revealed that the phosphatidylserine eversion rate in LM3 cells transfected with 1 μg microRNA-497 was significantly higher than that in miRNA group (P = 0.017) (Figure 2).
MicroRNA-497 regulates XIAP in hepatic cancer

As shown in Figure 3, compared with LM3 cells transfected by 0.5 μg miRNA, LM3 transfected by 0.5 μg microRNA-497 presented markedly higher caspase-3 level.

MicroRNA-497 declined XIAP protein expression in LM3 cells

Western blot was performed to test XIAP protein expression in LM3 cells. As shown in Figure 4, XIAP protein expression was significantly reduced in LM3 transfected with microRNA-497 compared with that transfected by miRNA.

XIAP siRNA enhanced LM3 cell apoptosis induced by microRNA-497

To evaluate the influence of XIAP on LM3 cell apoptosis induced by microRNA-497, we used siRNA technique to downregulate XIAP protein level and then transfected microRNA-497 to LM3 cells.

Western blot demonstrated that XIAP siRNA obviously declined XIAP expression in LM3 cells (Figure 5A). Caspase-3 activity detection revealed that after transfected by microRNA-497, LM3 cells treated by XIAP siRNA exhibited significantly higher apoptosis level compared with control (P = 0.0085) (Figure 5B).

XIAP overexpression suppressed microRNA-497 induced LM3 cell apoptosis

To evaluate the effect of XIAP overexpression on LM3 cell apoptosis induced by microRNA-497, we used XIAP plasmid to upregulate XIAP protein level and then transfected microRNA-497 to LM3 cells.

Western blot demonstrated that XIAP plasmid markedly enhanced XIAP expression in LM3 cells (Figure 6A). Caspase-3 activity detection revealed that after transfected by microRNA-497, LM3 cells treated by XIAP plasmid presented significantly lower apoptosis level compared with control (P = 0.0082) (Figure 6B).

Discussion

This study used hepatic cancer cell line LM3 to investigate the role of microRNA-497 on LM3 cells and related mechanism from the molecules and protein levels. It was showed that microRNA-497 significantly declined human hepatic cancer cell line LM3 cell proliferation and induced cell apoptosis, which was in accor-
MicroRNA-497 regulates XIAP in hepatic cancer

Previously, there are few reports about the impact of microRNA on hepatic cancer [3]. MicroRNA-218 can inhibit hepatic cancer growth, whereas microRNA-34a is associated with tumor metastasis [13, 14], suggesting that microRNA may also participate in the occurrence and development of hepatic cancer [13-15]. MicroRNA was reported to be closely related to tumor occurrence and development [23, 24], but the specific mechanism was unclear. This study discussed the relationship of microRNAs with apoptosis protein family.

XIAP protein is a type of anti-apoptosis protein [23]. Whether XIAP can be regulated by microRNA-497 and further affect LM3 cell proliferation was still controversy [24, 25]. Our results showed that microRNA-497 transfection decreased XIAP level in LM3. Moreover, LM3 apoptosis enhanced after microRNA-497 and XIAP siRNA transfection, while XIAP plasmid transfection suppressed microRNA-497 induced LM3 cell apoptosis.

Our results proved the effect of XIAP protein in microRNA-497 induced LM3 cell apoptosis. Western blot demonstrated that XIAP protein obviously declined after microRNA-497 transfection. After XIAP siRNA transfection, LM3 cell apoptosis markedly enhanced after microRNA-497 transfection. On the contrary, XIAP plasmid transfection obviously suppressed LM3 cell apoptosis induced by microRNA-497 transfection.

XIAP protein plays an important role in microRNA-497 induced LM3 cell apoptosis, suggesting that XIAP protein may be treated as a new strategy for hepatic cancer molecular targeted therapy [26]. At present, XIAP also showed apoptosis inhibition effect in other types of cancer [22, 24, 27, 28]. However, there is still no report about the regulatory relationship between microRNA-497 and XIAP in hepatic cancer. This is the first study to elucidate their relationship in hepatic cancer.

Conclusion

MicroRNA-497 restrained hepatic cancer cell line LM3 cell proliferation and triggered cell apoptosis by inhibiting XIAP, revealing that XIAP might be a potential target for hepatic cancer treatment.

Disclosure of conflict of interest

None.

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References

microRNA-497 regulates XIAP in hepatic cancer


