

Original Article

IL-17A enhance the effectiveness of IFN- α to regulated miR-23b in HepG2.2.15 cells

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Abstract: Hepatitis B virus (HBV) mainly damage liver cells through immune response. The purpose of this study was to determine whether the expression of MicroRNAs-23b (miR-23b) would be regulated by interferon- α and judge the value to antiviral treatment. Line of Hepa2.2.15 cells were cultured with interleukin-17A (IL-17A), tumor growth factor- β 1 (TFG- β 1) and interferon- α (INF- α), detect the expression of miR-23b in culture solution. We found when co-cultured with IL-17A and interferon- α , the duration of miR-23b peak concentration was pro-longed significantly in cell culture medium. In Conclusion, this study showed that INF- α can up-regulate the expression of miR-23b, IL-17A and TGF- β 1 would enhance the effectiveness.

Keywords: miR-23b, interferon- α , IL-17A, TGF- β 1, HepG2.2.15

Introduction

Hepatitis B virus (HBV) is the cause of the Chronic Hepatitis B (CHB), which can progress to hepatocellular carcinoma (HCC). The risk of HCC has been largely driven by chronic infection of hepatitis B and/or C viruses over the past century, and it accounts for 70-85% of the total liver cancer [1, 2].

An increasing number of literatures show that the liver cells damaged by HBV through immune response [3], the expression of T help (TH) cells and the inflammatory factors secreted by TH cells both changed in CHB.

MicroRNAs (miRNAs) are a class of small (consist of 18 to 25 nucleotides), endogenous, non-coding RNAs with a fundamental role in the regulation of gene expression [4], which through control various biological processes, including cellular development, apoptosis, proliferation, differentiation, and tumorigenesis [5] to have a central role in the development and regulation of the immune system [6, 7].

Zhu S' group have confirmed that miR-23b could suppresses autoimmune pathogenesis

and the interleukin-17 (IL-17) is responsible for miR-23b regulation [8]. The results of previous studies have found that the balance between regulatory T (Treg) cells and IL-17+ T helper (Th17) cells was changed in the patients with chronic HBV infection, and the level of IL-17 was up-regulated at the same time [9, 10]. However, the relationship between miR-23b and IL-17A in chronic HBV infection disease is unclear.

Here, we studied the expression of miR-23b in HepG2.2.15 cells. The purpose of this study was to determine whether the expression of miR-23b would be regulated by interferon- α (INF- α) or IL-17A and to judge the value of these indicators to antiviral treatment.

Materials and methods

Cell culture

Line of HepG2.2.15 cells was received from Dr. Zhu (Shanghai Jiao Tong University). IL-17A (2 ug, R&D system, US) and tumor growth factor- β 1 (TFG- β 1, 2 ug, R&D system, US) both were reconstituted to 20 ng/ml with sterile 4 mM HCL and at 2 ng/ml concentration, IFN α -1b diluted to 1 MIU/ml with DMEM and at 100 u/ml concentration.

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Table 1. The results of the expression of miR-23b in HepG2.2.15 cells

	0 H	24 H	48 H	72 H
INF- α	6.42 \pm 1.42	10.9 \pm 0.24*	4.94 \pm 0.29	11.94 \pm 0.16**
IL-17A	6.42 \pm 1.42	10.18 \pm 1.98*	9.17 \pm 0.17	8.79 \pm 0.14
TGF- β 1	6.42 \pm 1.42	9.64 \pm 0.5*	6.4 \pm 0.78	8.37 \pm 0.35
INF- α +IL-17A	6.42 \pm 1.42	6.71 \pm 2.00*	7.27 \pm 0.17*	12.00 \pm 0.14**
INF- α +TGF- β 1	6.42 \pm 1.42	6.88 \pm 2.22	7.65 \pm 0.77	11.26 \pm 0.47**

Interferon- α (INF- α), Interleukin-17A (IL-17A), Tumor Growth Factor- β 1 (TGF- β 1), *P<0.05, **P<0.01.

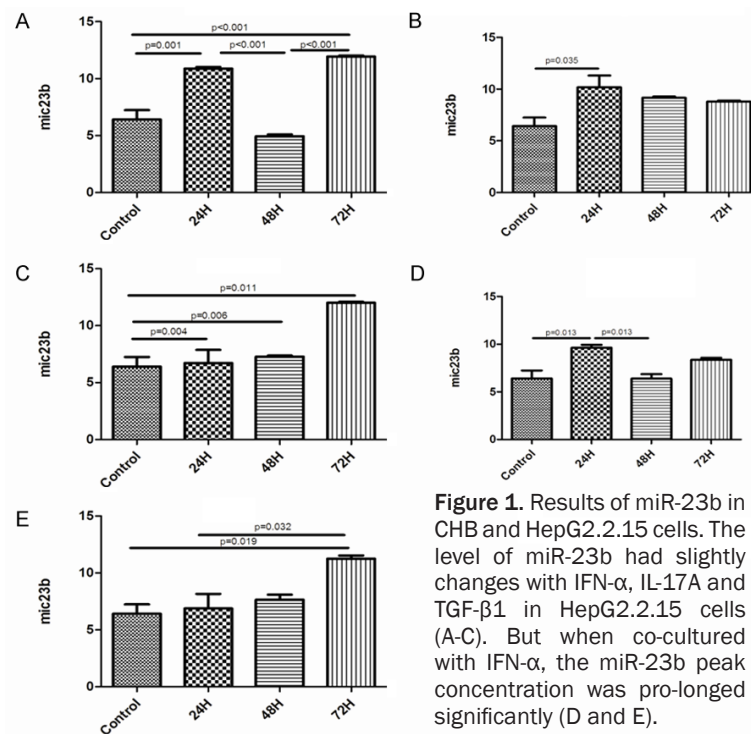


Figure 1. Results of miR-23b in CHB and HepG2.2.15 cells. The level of miR-23b had slightly changes with IFN- α , IL-17A and TGF- β 1 in HepG2.2.15 cells (A-C). But when co-cultured with IFN- α , the miR-23b peak concentration was pro-longed significantly (D and E).

The cells were grown in DMEM medium supplemented with 10% fetal bovine serum and maintained in a humidified incubator (5% CO₂) at 37°C.

All cultures were added with IL-17A, TGF- β 1 and IFN α -1b for 72 hours respectively, and changed the cultures at 48 hours. Then detect the culture mediums in 0, 24, 48 and 72 hours.

Quantitative real-time reverse transcriptas-epolymerase chain reaction

Stem-loop reverse transcriptase-polymerase chain reaction for miR-23b (P/N 000400 Applied Biosystems) were used to quantify miRNAs according to previously published conditions [11]. and U6 was used to normalize data

in miRNA quantification (P/N 001973, Applied Biosystems). The Δ threshold count method was used to calculate the fold change.

Statistical analysis

Results were reported as mean \pm standard. The one-way ANOVA method was used for multiple comparisons. Data analysis was done by using SPSS version 13.0 for Windows, Confidence interval was 95%.

Results

IFN- α raised the level of miR-23b in HepG2.2.15 cells

Brought up with IFN- α , the level of miR-23b in HepG2.2.15 was significantly raised in culture from (6.42 \pm 1.42) to (10.9 \pm 0.24) in 24 hours and returned to (4.94 \pm 0.29) in 48 hours, when changed the culture medium, the level increased again (11.94 \pm 0.16) after 72 hours (Table 1 and Figure 1A).

IL-17A added with IFN- α could prolong the expression time of miR-23b in HepG2.2.15 cells

Although IL-17A could slightly raise the level of miR-23b (10.18 \pm 1.98) in 24 hours (Table 1 and Figure 1B), but when added IFN- α to cell-culture medium, the action time of miR-23b would be prolonged from (7.27 \pm 0.17) in 24 hours to (12.00 \pm 0.14) at the third day (Table 1 and Figure 1C).

TGF- β 1 added with IFN- α would also prolong the action time of miR-23b in HepG2.2.15 cells

Like IL-17A, the level of miR-23b raised by TGF- β 1 separately would transiently in 24 hours (9.64 \pm 0.5) (Table 1 and Figure 1D), but when added with IFN- α , it could delay the top time of miR-23b to 72 hours (Table 1 and Figure 1E).

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The data shown that when co-cultured with IL-17A or TGF- β 1, the expression of miR-23b in HepG2.2.15 cells was significantly prolonged than use IFN- α cultured separately.

Discussion

Chronic HBV infection can cause autoimmune reactions to damage the liver cells. Many CHB related research shows that there are significantly changes in immune cells and which correlated with the degree of inflammatory activity and liver pathology HAI score [12].

There are studies have proved that miR-23b could by acting on multiple targets, including SPRY, ANXA2, ARHGEF6, CFL2, LIMK2, PIK3R3, PLAU, VEGFC, PTPN9, PTPRF, TAB2, TAB3 and IKK- α [8, 13-16] to suppress the development and pathogenesis of multiple autoimmune diseases, or regulated the proto-oncogene to inhibit proliferation, migration and invasion, and induces cell cycle arrest and apoptosis directly [17, 18].

Interferon- α is widely used to treat CHB for the function of immunoregulation [19, 20]. We stimulated the HepG2.2.15 cells with IFN- α , the level of miR-23b in culture medium was increased significantly in 24 hours and decreased at the next day, it maybe due to the effectiveness of common IFN- α could last 48 hours. So when replaced the fresh IFN-containing medium, the expression of miRNA-23b rise again.

Th17 cells, which are directly involved in and mediate chronic inflammation, have been confirmed the expression of active in chronic HBV infection. IL-17A, as the main inflammatory factor of TH17 cells, could regulate immunoinflammatory responses through miR-23b [9].

IL-17A is responsible for miR-23b regulation and many reports have shown the level of IL-17A was raised in CHB. We used IL-17A and IFN- α to stimulate the HepG2.2.15 cells separately. The expression of miR-23b was slightly changed with IL-17A. But when co-cultured with IL-17A and IFN- α , there was a distinctly change in miR-23b, the duration of miR-23b peak concentration was pro-longed significantly in cell culture medium. Those data exposed that IL-17A may enhance the effectiveness of IFN- α to up-regulated miR-23b.

Since TH-17 cells can cause inflammation and host antimicrobial immunity, Foxp3+CD4+CD25+ regulatory T cells (Foxp3+ Treg) is played an anti-inflammatory role to against it. We further to culture the HepG2.2.15 cells with TGF- β 1, an important inflammatory factor secreted by Treg cells. Results show that similar to IL-17A, the level of miR-23b was prolonged by coefficient with TGF- β 1 and IFN- α . It prompted that TGF- β 1 may also enhance the effectiveness of IFN- α . The data pointed out that IL-17A and TGF- β 1 could maintain the stability of the level of miR-23b stimulated by IFN- α , which maybe lead to enhance the antiviral effect by IFN- α .

In conclusion, this study highlighted that IFN- α can up-regulate the expression of miR-23b, IL-17A and TGF- β 1 would enhance the effectiveness. As we did not perform functional studies to understand the relationship between miR-23b and IFN- α . We will further investigate the change of miR-23b in CHB with IFN- α therapy. Also we will design cell experiment to conform the pathways between miR-23b and IFN- α .

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Disclosure of conflict of interest

None.

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