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Original Article

Metadherin interference inhibits proliferation and enhances chemo-sensitivity to doxorubicin in diffuse large B cell lymphoma

Pei-Pei Li[1], Li-Li Feng[1], Na Chen[1], Kang Lu[1], Xiao-Hui Meng[2], Xue-Ling Ge[1], Xiao Lv[1], Xin Wang[1,2]

[1]Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250012, Shandong, China; [2]Institute of Diagnostics, School of Medicine, Shandong University, Jinan 250012, Shandong, China

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Abstract: Metadherin (MTDH) is highly expressed in many tumors and is involved in the proliferation, metastasis and drug resistance of tumor cells by regulating multiple signaling pathways. Our previous studies demonstrated that MTDH is overexpressed in diffuse large B cell lymphoma (DLBCL) and involved in apoptosis resistance, in part, via Wnt signaling. Here, we investigated the role of MTDH in the chemo-sensitivity of DLBCL. The study was performed in the DLBCL cell line LY8 to investigate the relationship between MTDH expression and doxorubicin (DOX) sensitivity in DLBCL. A MTDH interference model was developed in LY8 cells by transfected with lentivirus which is carrying MTDH interference sequence. Western blot was used to detect the protein expression. A CCK-8 assay was used to evaluate cell proliferation. The results showed that DOX treatment had no effect on the intracellular MTDH expression of LY8 cells. The proliferation of LY8 cells was inhibited after MTDH interference. MTDH interference increased the DOX sensitivity in the LY8 cell lines. The results suggested that MTDH is a potential therapeutic target in DLBCL, and it cooperates with DOX in treatment of DLBCL.

Keywords: Diffuse large B cell lymphoma, metadherin, doxorubicin, chemo-resistance, therapeutic

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL) in adults, accounting for 30%-40% of NHL [1, 2]. DLBCL can be divided into three subtypes, which include germinal center B-like DLBCL (GC-like DLBCL), activated B-cell-like DLBCL (ABC-like DLBCL) and the third type [3]. Currently, the standard treatments for DLBCL include the anthracycline-based combinatorial chemotherapy regimens R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone); when used as the first-line treatment regimen, these compounds have an approximately 60% cure rate [4]. Despite the improvements induced by the successful therapeutic effects of R-CHOP, patients who experience relapse and a refractory disease remain a challenge in the treatment of this disease [5, 6]. Gene-expression analyses have revealed many different pathways that resulted in drug resistance in DLBCL. Further elaborating the molecular pathways and the associated resistance genes may contribute to the discovery of new therapeutic targets and would ultimately benefit the prognosis of DLBCL patients [7, 8].

Metadherin (MTDH) was first found in astrocyte cells, which were treated with tumor necrosis factor (TNF)-α or infected with human immunodeficiency virus (HIV) [9, 10]. MTDH was expressed in nearly all tumor cells and promoted tumors via a variety of mechanisms involved in tumor cell proliferation, survival and metastasis activity. Recently, MTDH was associated with the chemo-sensitivity and chemo-resistance of tumor cells by regulating series genes [11-15]. This effect resulted from the activation of phosphatidylinositol 3-kinase (PI3K)/AKT and nuclear factor (NF)-κB and increased multi-drug resistance (MDR1) translation by MTDH [13, 16, 17].

Our previous study demonstrated that MTDH is overexpressed in DLBCL primary cells and the DLBCL cell line LY8, which was associated with the apoptosis deregulation of DLBCL [18]. Here,

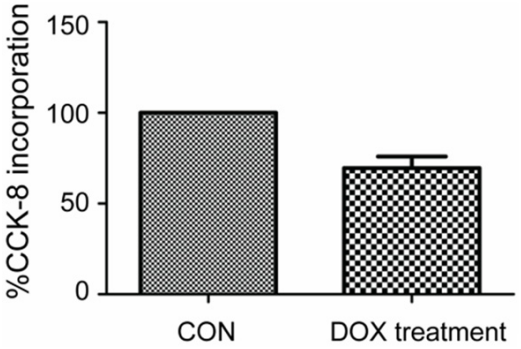

Figure 1. Proliferation change in LY8 after DOX treatment for 24 h. Compared to the CON without DOX treatment, proliferation in LY8 cells with DOX (0.1 ng/ml) treatment for 24 h was inhibited by approximately 30%.

we investigated its effect on proliferation in DLBCL and its role in DOX treatment in DLBCL using the LY8 cell line.

Materials and methods

Cell line culture and DOX treatment

The human DLBCL cell line LY8 was maintained in Iscove's Modified Dulbecco's Medium (IMDM; Hyclone, Logan, UT, USA) supplemented with 10% fetal calf serum (FBS, Hyclone, Logan, UT, USA) at 37°C and 5% carbon dioxide.

Western blot analyses

The proteins were lysed by RIPA containing 1% phenylmethanesulfonyl fluoride (PMSF) (Shenergy Biocolor, Shanghai, China). A BCA assay was used to detect the protein concentration. Forty μg of protein underwent 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were electrophoresed on a subsequent transfer membrane for 90 min under a voltage of 100 V. This was followed blocking by 5% milk at room temperature for 1 hour and then incubated with antibodies at 4°C for overnight. The concentration of the antibodies used in this study was as follows: anti-MTDH 1:250 (Invitrogen, Frederick, MD), anti-β-actin 1:2000 (Zhongshan Goldenbridge, Beijing, China). The next day, the samples and the secondary antibody were incubated for 30 min after the membrane was washed three times and developed using Multi Gauge Ver.4.0 software for analysis. The experiment was repeated three times.

Small interference RNAs

siRNAs that targeted MTDH and a control scrambled siRNA were synthesized by Shanghai Genechem Co. The sequencing was designed according to other reports, and the efficacy was detected by our previous studies [12, 18, 19]. The methods were as followed: 10^4 LY8 cells were plated into 96-wells culture plates and co-cultured with lentivirus with sequences of siRNA that interfered with the MTDH gene or a negative control in the total culture volume of 100 μl for 10 hours. The multiplicity of infection (MOI) was 100. The interfering efficiency in LY8 was determined by fluorescence microscope and flow cytometry after three days of transfection.

Proliferation assays

Seven days after transfection, 5×10^3 cells from different sources of subgroup species were plated in 96-well plates. Each sample was repeated three times. The culture system was 100 μl. After 24 hours in culture, 10 μl of CCK-8 reagent were added to the system for the detection of proliferation. After 4 hours of co-culture with CCK-8, the absorbances were detected at 450 nm wavelength.

Statistical methods

SPSS18.0 software for windows was used for data analysis. All data were analyzed by 2-tailed Student's t tests, $P<0.05$.

Results

DOX inhibited the proliferation of LY8 and had no effect on MTDH expression

DOX is a basic therapeutic agent in DLBCL treatment. According to the literature, 0.1 ng/ml was chosen as the terminal concentration of DOX [20]. After treatment with DOX for 24 hours, a CCK-8 assay was used to detect the proliferation activity of the LY8 cell line. The results showed that the proliferation of LY8 was inhibited by approximately 30% ($P<0.05$) (**Figure 1**).

Then, we investigated whether DOX treatment reduced the expression of MTDH in protein levels. The results showed after 24 hours in cultures with DOX, the expression of MTDH in LY8

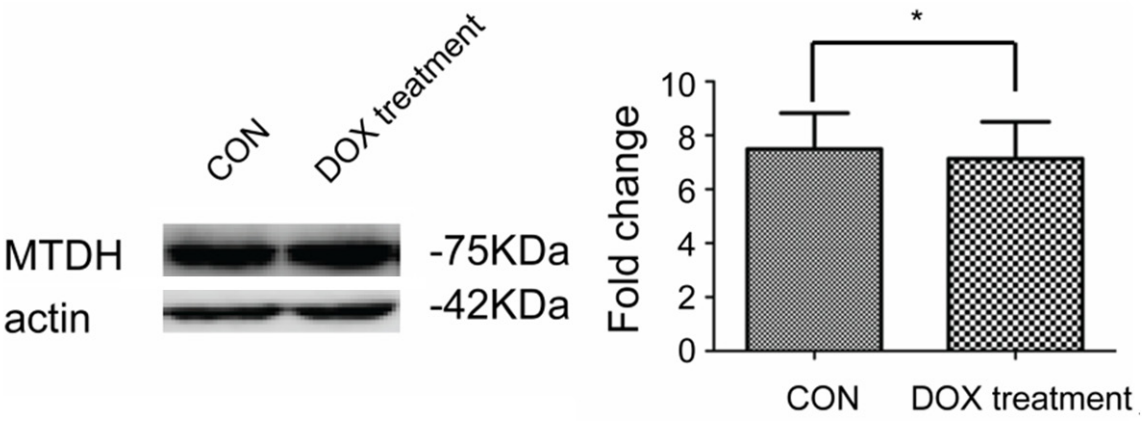

Figure 2. MTDH protein levels after treatment with DOX. After treatment with DOX for 24 h, there was no obvious change in the MTDH protein level. The studies were repeated three times, *P<0.05.

was not significantly changed (P>0.05) (**Figure 2**).

MTDH interference inhibited proliferation of LY8

The CCK-8 method was used to evaluate the proliferation change in the following studies. Lentivirus that contained a MTDH interference sequence or a negative control sequence was used to infect the LY8 cell lines. The transfection efficiency of the cell lines in this part of the study was evaluated by fluorescence microscopy and flow cytometry. The transfection efficiency was approximately 78% (**Figure 3**). Furthermore, the transfection efficiency was consistent with our previous results [18]. In this condition, the MTDH protein level in the MTDH interference LY8 cells was downregulated approximately 60% [18]. The CCK-8 method was then used to detect the proliferation changes in LY8 cells. After MTDH interference, the proliferation of LY8 cells was inhibited about 34% (**Figure 4**).

MTDH interference enhanced DOX sensitivity

Then, we investigated whether MTDH interference enhanced the sensitivity of DOX in the LY8 cells. The CCK-8 method was used to evaluate the change in proliferation. The results showed that MTDH interference further enhanced the sensitivity of LY8 to DOX treatment, and the proliferation rate was further downregulated by approximately 26% (**Figure 4**).

Discussion

Our results showed that MTDH can promote the proliferation activity in DLBCL. Furthermore, MTDH can mediate the chemo-sensitivity of DOX in the DLBCL cell line LY8.

DOX was treated as a basic therapeutic agent in DLBCL. Clinically, clear differences exist in the prognosis of GCB-type and ABC-type DLBCL [3]. The traditional opinion insists that the activity of NF-κB signaling pathway is one of the most important features of ABC-type DLBCL. Hoverer, in the GCB-type DLBCL cell line LY8, the activity of the NF-κB pathway was upregulated [20, 21]. Furthermore, the ABC-type DLBCL cell lines showed increased sensitivity to DOX treatment compared with the GCB-type in vitro, which is different from the result of patients with DLBCL after therapy in a clinical trial [20]. Thus, we chose the LY8 cell line, which belongs to the GCB-type DLBCL and has an intermediate sensitivity to DOX treatment, to evaluate the effects of MTDH in DOX chemo-sensitive cells.

Our data show that DOX at a concentration of 0.1 ng/ml inhibited 30% proliferation of LY8 after 24 h of treatment. There was no effect on MTDH expression during DOX treatment. In our previous study, we demonstrated that MTDH interference induced apoptosis of the LY8 cell line [18]. Here, we further evaluated the proliferation changes in the LY8 cell line. The results showed that MTDH interference inhibited the proliferation of LY8 cells. In MTDH interference

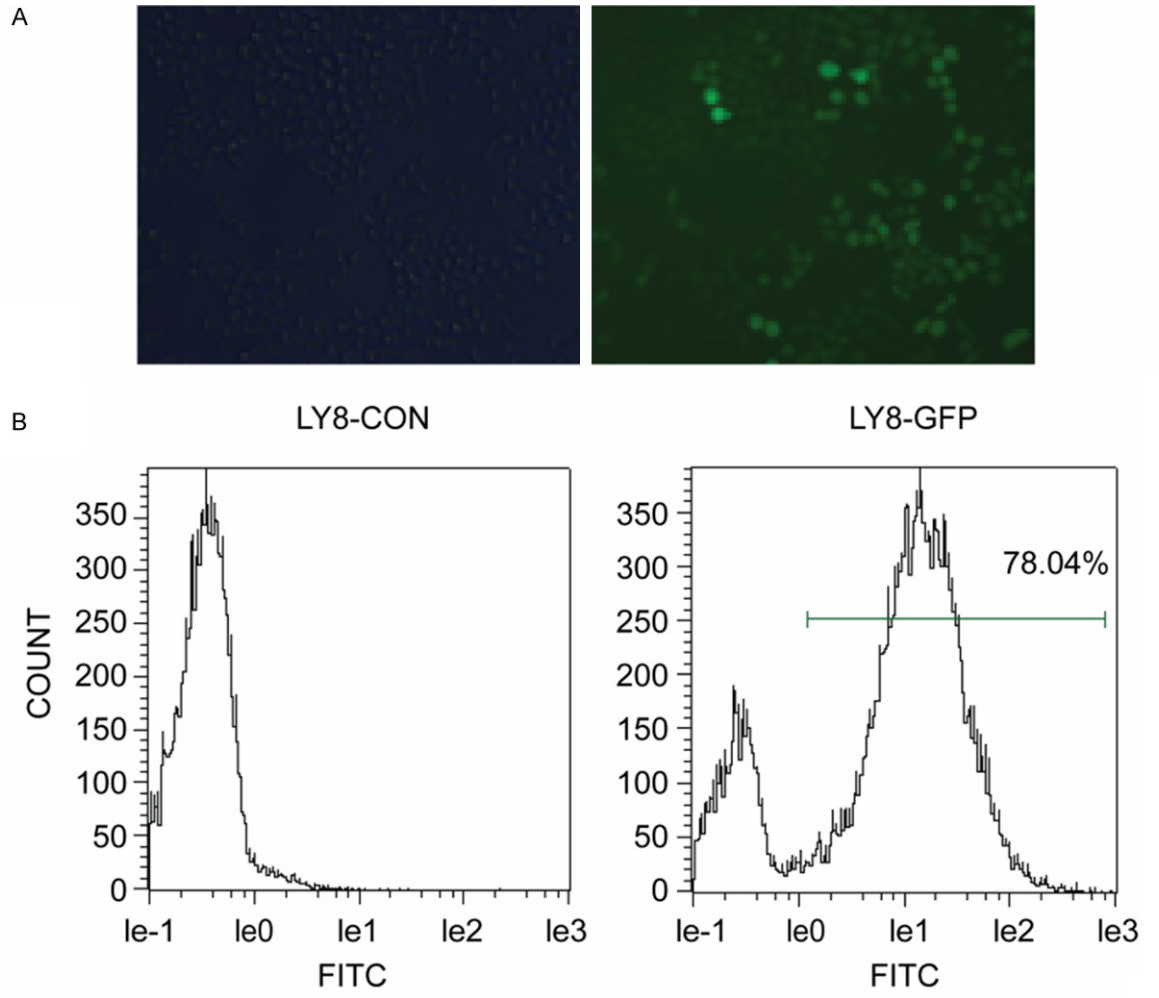

Figure 3. Transfection efficiency of LY8 cells. A. Fluorescence microscopy images showing lentivirus-infected cells. B. GFP expression in LY8 cells infected with lentivirus as determined by flow cytometry.

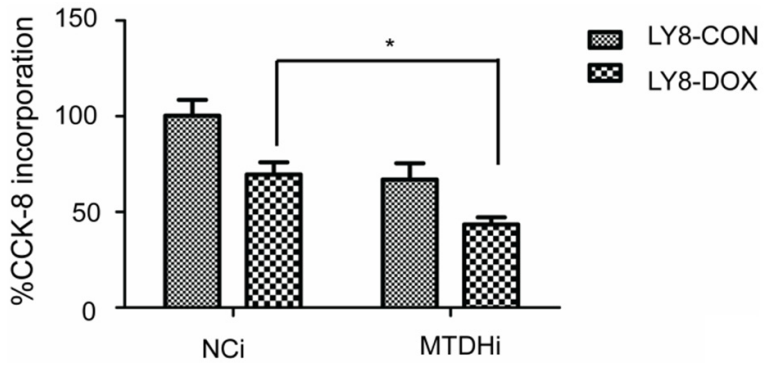

Figure 4. MTDH interference enhanced the chemo-sensitivity of DOX in LY8 cells. The proliferation rate in LY8 cells infected with lentivirus that contained the MTDH interference sequence (mtdhi) or a negative control sequence (nci) was 69.55±6.335 vs 43.46±3.694, respectively, **P*<0.05.

cells, DOX had greater inhibitor activity in proliferation of LY8 cells. This finding indicated that MTDH interference is beneficial to DOX treatment in DLBCL.

DOX leads to DNA double strand breaks, which affects the cell cycle control, DNA repair and cell death. It is broadly used in the chemotherapeutic regimen of many malignancies, such as small cell lung cancer, breast cancer and lymphoma [22-24]. In other tumors, such as ovarian cancer and hepatic cancer, the inhibition activity of the PI3K or NF-κB pathways can enhance the sensitivity of the chemotherapy of DOX [25-27]. MTDH has been shown to be involved in promoting chemo-sensitivity

and reversing the state of multidrug resistance of chemo-resistance to DOX in hepatic cancer, breast cancer and neuroblastoma via PI3K and NF-κB [28-30]. DLBCL has also been demonstrated to protect tumors cells from death induced by chemotherapy, in part, through the activation of the PI3K or NF-κB pathways [20, 31, 32]. This may be the downstream mechanism of MTDH, which promotes the sensitivity of DOX in DLBCL.

Therefore, our study results elucidate the functional significance of MTDH in the biology of DLBCL, and its role in regulating the chemo-sensitivity of DOX. Further research should focus on the concise mechanism of MTDH that enhances chemo-sensitivity. The relapse and refractory models should also be used to further investigate whether MTDH is involved in the chemo-resistance of DLBCL. These results may elucidate novel therapeutic targets in DLBCL and a potential therapeutic strategy to overcome drug resistance.

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Disclosure of conflict of interest

None

Address correspondence to: Dr. Xin Wang, Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong University, Add: No. 324, Jingwu Road, Jinan 250021, Shandong, China. Tel: 0086-0531-85188331 (B); 0086-13156012606 (M); Fax: 0086-0531-87061197 (B); E-mail: xinw-007@126.com

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