MicroRNA-24-3p regulates Hodgkin's lymphoma cell proliferation, migration and invasion by targeting DEDD

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Abstract. Hodgkin's lymphoma (HL) is a common hematological tumor, and the incidence is increasing. At present, it is considered that miRNAs are closely related to HL. Substantial attention has been paid to the effects of miRNA on the pathophysiological process of HL. This study was focused on the potential role of miR-24-3p in HL by targeting DEDD. The reverse transcription-quantitative PCR (RT-qPCR) results demonstrated that miR-24-3p expression was highly elevated and DEDD expression reduced inversely in HL tissues compared to adjacent tissues. According to the results of CKK-8 assays, miR-24-3p was able to accelerate HL cell proliferation. In addition, the results of the Transwell assays also indicated that miR-24-3p promoted the invasion and migration abilities of HL cells. Moreover, the results demonstrated that miR-24-3p inhibited DEDD expression. Hence, the present study revealed that miR-24-3p could accelerate HL development through inhibiting DEDD.

Introduction

Hodgkin's lymphoma (HL) is a common lymphatic tumor, and the incidence rate by year of HL new cases range from three to four per 100,000 western individuals (1). HL is an malignant tumor derived from B cells (2). As it is hard to diagnose advanced HL, and the pathophysiological mechanisms of HL remain unknown, approximately 20% of the newly diagnosed cases die from HL (3). Consequently, more effective treatments to increase HL survival rates need to be developed. At the same time, understanding of the mechanisms of HL development is required. Studies of factors associated with HL, such as genes and miRNAs, are also important.

miRNAs are 19-23 nucleotides in length. As a subgroup of non-coding miRNAs, they play a role in regulating post-transcriptionally via targeting mRNA 3'UTR, causing their degradation or suppressing mRNA translation (4-6). An increasing number of studies have emerged, providing evidence that various miRNAs can exert oncogenic or tumor suppressive functions (7,8). For example, miR-148a suppresses migration and invasion of breast cancer (9). miRNA-29b suppresses tumor angiogenesis, invasion, and metastasis (10). miR-24-3p was proven to be involved in multiple kinds of tumors, including HL (11), head and neck squamous cell cancer (12), and bladder carcinoma (13). However, the function of miR-24-3p in mediating HL invasion and migration remains largely elusive.

The death effector domain-containing protein (DEDD) has a relevance to different kinds of physiological processes, including cell mitosis, cycle and apoptosis (14). Studies have shown that DEDD may be used as a potential therapeutic target and prognostic marker to cure metastasis of carcinoma (15,16). However, the effects of DEDD on regulating HL have not yet been reported. To better understand the anti-metastatic functions and mechanisms of DEDD in HL, the present study investigated the DEDD expression levels in HL tissues and cell lines. At the same time, the correlation between miR-24-3p and DEDD in HL was analyzed.

Materials and methods

Cell cultures. HL cell lines [L1236 (CSC-C0538, Creative Bioarray) and L428 (ws101345, ATCC)] were cultured in RPMI-1640 medium (Cellgro; Corning, Inc., Corning, NY, USA) in an atmosphere with 5% CO2 at 37˚C. The medium contained penicillin/streptomycin, 5% L428, 10% L1236 as well as 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Germinal center (GC)-B cells were sorted from tonsil tissue samples of three HL donors aged between 3 and 10 years. Two of the three GC-B cells were purified >98% from human tonsil tissues on the basis of the CD20+lgD-CD38+ expression, as previously described. The third sample was magnetic-activated cell sorting purified >95% based on expression of IgD CD138 CD3'CD10'. Written informed consents were obtained for the use of the tonsil samples from the parents of the children.

Tissue specimens. We collected the HL patients' tonsil tissue samples and matched normal tissues from the First Affiliated
miR-24-3p expression is upregulated and DEDD expression is downregulated in HL. To evaluate miR-24-3p and DEDD expression levels in HL, we collected HL tissues and matched normal tissues from 30 HL patients, and then measured the DEDD expression as well as miR-24-3p expression. The results of RT-qPCR revealed that the miR-24-3p expression level in HL was significantly increased in contrast with that in the normal control tissues. Moreover, we found that the DEDD expression was decreased compared with that in normal tissues and cultured L1236 and L428 cells respectively.

**Statistical analysis.** All the above experiments were performed 3 times. The statistical analysis was evaluated by the GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) together with SPSS 18.0 version (SPSS, Inc., Chicago, IL, USA). Student’s t test and one way ANOVA followed by Tukey’s post hoc test were used to analyze two or multiple groups, respectively. Statistically significant difference was set at P<0.05.

**Results.**

miR-24-3p expression was upregulated and DEDD expression was downregulated in HL. To evaluate miR-24-3p and DEDD expression levels in HL, we collected HL tissues and matched normal tissues from 30 HL patients, and then measured DEDD expression as well as miR-24-3p expression. The results of RT-qPCR revealed that the miR-24-3p expression in HL tissues was significantly increased in contrast with that in the normal control tonsil tissues (Fig. 1A and B, P<0.01). Moreover, we found that the miR-24-3p expression level in GC-B cells was decreased compared with that in HL cells.
miR-24-3p accelerates HL cell proliferation. The HL cells (L1236 and L428) transfected with mimics or inhibitor of miR-24-3p were used for detecting the role of miR-24-3p in HL cell proliferation. The results of RT-qPCR assays demonstrated that the miR-24-3p mimic expression was high in L1236 and L428 cells (Fig. 2A and B, P<0.001 and <0.01). Subsequently, the CCK-8 assay was conducted to observe HL cell proliferation ability, and the results demonstrated that miR-24-3p promoted L1236 cell (Fig. 2C) and L428 cell (Fig. 2D) proliferation.

miR-24-3p accelerates HL cell invasion and migration. We studied cell invasion and migration abilities in HL cells which were transfected with mimics or inhibitor of miR-24-3p. The results showed that there was a significant rise in the invasion of L136 and L428 cells transfected with mimics of miR-24-3p in contrast with the HL cells transfected with control mimics (P<0.001). On the contrary, the Transwell results demonstrated a significant decrease in the invasion of L136 and L428 cells transfected with inhibitor of miR-24-3p compared to the control group (Fig. 3A and C, P<0.001 and <0.01). In addition, according to the Transwell assays, the results also demonstrated that miR-24-3p promoted HL migration ability (Fig. 3B and D, P<0.001 and <0.01).

miR-24-3p suppresses DEDD gene transcription in HL by targeting its 3’UTR. To investigate whether DEDD expression had relevance to miR-24-3p expression and to better understand the function of miR-24-3p in HL, TargetScan was used to find the target sites in the DEDD sequence of miR-24-3p (Fig. 4A). We measured the DEDD 3’UTR luciferase activities by performing luciferase reporter gene assays. We co-transfected mimics of miR-24-3p and wild-type DEDD 3’UTR vector or mutant DEDD 3’UTR vector into HL cells to observe whether DEDD was the target of miR-24-3p, and then detected the role of miR-24-3p in regulating the mRNA and protein expression of DEDD. The results showed that there was a significant decrease of fluorescence activity in both L1236 (P<0.01) and L428 (P<0.001) cells co-transfected with the wild-type DEDD
3'UTR vector and miR-24-3p in contrast with the control group, however, between cells co-transfected with the mutant DEDD 3'UTR vector and miR-24-3p and the control group, there was no significant difference (Fig. 4B and C).
of RT-qPCR and western blotting both demonstrated that miR-24-3p could inhibit DEDD expression in L1236 and L428 cells (Fig. 4D and E, P<0.001 and <0.01).

The role of DEDD in regulating miR-24-3p effects in HL cell migration and invasion. We investigated whether DEDD was required in regulating miR-24-3p functions of promoting HL cell invasion and migration. Overexpression vector of DEDD and miR-24-3p mimics were co-transfected into L1236 and L428 cells using RT-qPCR to detect the DEDD mRNA expression, the results are shown in Fig. 5A. Then, we detected the migration and invasion in HL cells which were co-transfected with overexpression vector of DEDD and miR-24-3p mimics using Transwell assay. The results revealed that the overexpression of DEDD markedly reversed miR-24-3p-mediated promotion of cell migration and invasion in HL cells (Fig. 5B-D, P<0.01). Collectively, these data suggested that DEDD may reverse partial function of miR-24-3p in HL cells.

Discussion

HL is a particular B-cell malignancy, accounting for a large percentage of lymphomas (18). Despite significant advances in the treatment of HL, existing treatments fail to cure 10-20% of HL patients. Similarly, 10-20% of HL patients may be over-treated. So, reliable predictive biomarkers for HL patients who need intensive therapy remain a challenge. Such biomarkers could also provide insight into the biology of HL.

Several studies have indicated that different kinds of miRNAs are involved in different kinds of biological activities, for instance, cell proliferation, migration, metastasis and inflammation (19,20). Multiple studies have found that many different miRNAs play an important role in the development of HL. For example, miR-124a methylation is associated with aggressive HL disease (21); miR-374b can suppress cell proliferation and promote cell apoptosis by inhibiting AKT1 and Wnt-16 in T-cell lymphoblastic lymphoma (22); miR-9 methylation is a common event in HL and participated in HL pathogenesis (23); overexpression of miR-155 and inhibition of its target NIAM enhance cell growth in HL (24).

miR-24-3p is one of the most studied miRNAs in different kinds of tumors. Over the past decade, increasing evidence has indicated that dysregulation of miRNAs makes contributions to various aspects of tumorigenesis process. miR-24-3p is a master regulator in gene regulation and is related to diverse human disease (25). Several other studies also revealed an
oncogenic role for miR-24-3p in other types of cancer, for instance, miR-24-3p has been proved to promote glioma cell proliferation by targeting MXI1 (26); miR-24-3p represses small cell lung carcinoma VP16-DDP chemoresistance through ATG4A (27). However, as far as we know, there have been no previous studies which report the mechanism of miR-24-3p in HL. To explore the functional relevance of miR-24-3p in HL, we compared its expression level in HL tissues to that in paracarcinoma tissues. The results demonstrated that the miR-24-3p expression level in HL tissues was higher than that in paracarcinoma tissues. In addition, it also indicated that miR-24-3p could promote HL cell proliferation, invasion and migration through DEDD.

The present study provides evidence that miR-24-3p is involved in regulating HL cell proliferation, invasion and migration. At the same time, miR-24-3p was increased and that of DEDD expression was decreased in HL tissues. Moreover, miR-24-3p was able to suppress the gene transcription of DEDD. Hence, the present study demonstrated that miR-24-3p promoted HL progression via suppressing DEDD. Thus, it is suggested that miR-24-3p may be a key potential therapeutic target for the treatment of HL.

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Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions
JW drafted the manuscript. JW, KY and XLv were mainly devoted to cell culture. JW, QY and MS performed RT-qPCR. XLi and HS were responsible for western blotting. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). Signed informed consent was obtained from the parents of the patients.

Patient consent for publication
Not applicable.
Competing interests

The authors declare that they have no competing interests.

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