Leukemia Research 50 (2016) 104–111

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

DIXDC1 promotes tumor proliferation and cell adhesion mediated drug resistance (CAM-DR) via enhancing p-Akt in Non-Hodgkin's lymphomas

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ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 4 September 2016
Accepted 7 September 2016
Available online 8 September 2016

Keywords:
Non-Hodgkin's lymphomas (NHL)
DIXDC1
Proliferation
Cell adhesion
Drug resistance

ABSTRACT

DIX domain containing 1 (DIXDC1), is a human homolog of Ccd1, a DIX domain containing protein in zebrafish. The present study was undertaken to determine the expression and biologic function of DIXDC1 in Non-Hodgkin's lymphoma (NHL). Clinically, we detected that the expression of DIXDC1 was significantly lower in the indolent lymphomas compared with the progressive lymphomas by immunohistochemistry analysis. Functionally, we found that DIXDC1 could promote cell proliferation via modulating cell cycle progression and PI3K/AKT signaling pathway in NHLs. Moreover, we confirmed that DIXDC1 was involved in the process of lymphoma cell adhesion mediated drug resistance (CAM-DR). Adhesion to fibronectin (FN) or HS-5 up-regulated DIXDC1 expression, and up-regulation of DIXDC1 resulted in an increased expression of p-AKT, which promoted CAM-DR. Our finding supports the role of DIXDC1 in cell proliferation, cell cycle and CAM-DR in NHLs. We propose that inhibition of DIXDC1 expression may be a novel therapeutic approach for NHLs patients, and it may be a target for drug resistance.

1. Introduction

Non-Hodgkin's lymphoma (NHL) constitute heterogeneous group of malignant lymphoproliferative disorders displaying a diverse range of biological phenotypes, clinical behaviours and prognoses [1]. The incidence of NHL is rising steadily over recent years and remains associated with significant mortality [2,3]. Despite intensive effort in developing new therapies, there has been little progress in improving survival for patients, and the emergence of clinical drug resistance remains a barrier to successful treatment of NHL [4–6]. Therefore, it is necessary to explore mechanisms of drug resistance in NHL.

Many investigators have shown that lymphoma tumor microenvironment provides sanctuary for lymphoma cells through interaction occurs between the lymphoma cell and its microenvironment (lymph node stroma) [7,8]. Previous studies have shown that adhesion to fibronectin (FN) or bone marrow stromal cells is a critical reason for mediating tumor resistance to cytotoxic therapy (cell adhesion mediated drug resistance (CAM-DR)) [9]. CAM-DR has been described mainly for hematopoietic malignancies, especially in NHL [10,11] and multiple myeloma (MM) [12], as well as other types of cancer [13,14]. However, how the lymphoma microenvironment influences lymphoma cell survival and response to therapy, as well as the molecular mechanisms involved, remains unclear.

DIX domain containing 1 (DIXDC1), is a human homolog of Ccd1 and a positive regulator in the Wnt signaling pathway [15–17]. The DIXDC1 is involved in a variety of processes, such as cell proliferation, cell cycle, protein folding, transcription, protein transport, signal transduction. In the field of cancer research, up-regulated expression of DIXDC1 was first found in colorectal cancer and overexpression of DIXDC1 might target p21 and cyclin D1 to promote colon cancer cell proliferation and tumorigenesis [18]. Moreover, DIXDC1 was reported to increase the invasion and migration ability of non-small-cell lung cancer [19]. Recently study revealed that DIXDC1 could promote gastric cancer invasion and metastasis through the activation of the Wnt pathway [20]. These observations all revealed that DIXDC1 might function as an oncogene promoting tumor progression. However, the functional role of DIXDC in non-

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http://dx.doi.org/10.1016/j.leukres.2016.09.011
0145-2126/

Hodgkin's lymphoma (NHL) has never been elucidated. Considering the pivotal role DIXDC1 played in tumorigenicity control and the truth that DIXDC1 can regulate Akt activity, it would be interesting to verify whether DIXDC1 plays a key role in CAM-DR in NHL.

In this study, we aimed to investigate the expression and function of DIXDC1 in NHL and CAM-DR phenotype. Our study demonstrated for the first time that DIXDC1 is associated with clinical and pathologic factors in NHL. Furthermore, we also demonstrated that DIXDC1 plays a critical role in CAM-DR via enhancing p-Akt in NHL. Taken together, our study may provide a novel perspective for a better understanding of the role for DIXDC1in NHL and CAM-DR.

2. Materials and methods

2.1. Patients and specimens

A total of 46 indolent lymphoma (including 18 follicular lymphoma (FL), 28 extranodal lymphoma of mucosa-associated lymphoid tissue (MALT)), 66 progressive lymphoma (including 39 diffuse large B-cell lymphomas (DLBCL), 19 Burkitt lymphomas) and 20 reactive lymphoid (RL) tissues samples were obtained from the Department of Pathology, Affiliated Cancer Hospital of Nantong University (Nantong, China) from 1993 to 2005. The histological types of NHL cases were classified according to the World Health Organization (WHO) guidelines. The collection of NHL tissues was approved by the research ethics committee of the institute, and written informed consent was obtained from every patient. Tissues were fixed in 10% buffered formalin and embedded in paraffin for immunohistochemical study. Then, 2 reactive lymphoid and 6 pathologically confirmed DLBCLs fresh-frozen tissues from Affiliated Cancer Hospital of Nantong University were used for Western blot analysis.

2.2. Immunohistochemical staining

Immunoprecipitation was performed as described previously [21]. In short, slides were dewaxed by three 15 min and washed with xylene. Rehydration of tissues was performed by 5 min washes in 100%, 95%, and 80% ethanol and distilled water. Antigen retrieval was performed by heating the samples at 120 °C for 3 min in 10 mmol/L sodium citrate (pH 6.0). The tissue sections were incubated with anti-DIXDC1 (diluted 1:100; Santa Cruz Biotechnology) or Ki-67(diluted 1:100; Santa Cruz Biotechnology) for 4 h at room temperature. Antigen antibody reactions were visualized with DAB (Dako, Glostrup, Denmark). Tissue sections were then counterstained with hematoxylin, dehydrated and mounted.

2.3. Cell culture and preparation of transient transfection and siRNA

Burkitt lymphoma cell-line Daudi and diffuse large B cell lymphoma (DLBCL) cell lines OCI-Ly8 obtained from Fudan University (Shanghai, China). Bone marrow stromal cell line HS-5 obtained from Cell Library, China Academy of Science. The human lymphoma cell lines were grown in suspension in RPMI 1640 (Sigma-Aldrich, Rehovot) and the HS-5 was grown in DMEM (Sigma–Aldrich, Rehovot), supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY, USA) at 37 °C and 5% CO_2.

Full-length DIXDC1 cDNA were amplified using PCR and subcloned into pCMV-HA and pcDNA3.1 constructs [22]. The following primers were used:DIXDC1 forward, 5′-GGT GAT CCT CAT TCC AGT TTC CA-3′, DIXDC1reverse, 5′-AAT GCC ACC AGG CGA CAA TAC TA-3′. The control-siRNA and DIXDC1siRNA were obtained from Qiagen (cat. no. 1027020). The sequences of siRNA duplex targeting DIXDC1 are: sense, 5′-r AUG CCU UGC AGC AGA GAU dTdT-3′; antisense, 5′-r AUC UCU GCU GCA AGG CAU dCdC-3′. Transfection was performed using lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's instructions and transfected cells were used for subsequent experiments 48 h after transfection.

2.4. Cell co-culture

At first, dishes were coated with 40 mg/ml human FN (Sigma-Aldrich) or HS-5 cells at 37 °C. Then, lymphoma cells (10^5 cells/ml) were allowed to adhere to preincubated FN or HS-5 for 6 h and non-adherent cells were removed by five washes using washing buffer (DMEM + 0.1% BSA). Attached cells were incubated for 24 h at 37 °C. Finally, attached cells were carefully removed by washing buffer (PBS) for next experiments, with the HS-5 or FN monolayer kept intact.

2.5. Western blot analysis and antibodies

Western blot analysis was performed as described previously [23]. The following monoclonal antibodies were purchased from Santa Cruz Biotechnology.: anti-DIXDC1 (1:1000); anti-p21 (1:500); anti-Cyclin D1 (1:500); anti-Akt (1:1000), anti-Gsk-3β (1:1000), anti-p-Akt (1:500) and anti-p-Gsk-3β (1:500); anti-β-actin (1:1000Sigma); anti-myc (1:1000Sigma). Protein was run on a 10% PAGE and transferred to polyvinylidine difluoride filter (PVDF) membranes. The membranes were blocked with PBS containing 5% skim milk and 0.1% Tween-20 and then incubated with primary antibody overnight at 4 °C. After washing with PBS containing 0.1% Tween-20 three times, each for 5 min, filters were incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Values were responsible for at least three independent experiments.

2.6. Cell viability assay

The cells were seeded on a 96-well plate (Corning Inc., Corning, NY, USA) with a density of 1×10^6/well in volumes of 100 μL and grew 12 h with or without the addition of doxorubicin (Sigma–Aldrich, St. Louis, MO, USA). Then, cell proliferation was measured by Cell Counting Kit (CCK)-8 (Dojindo, Kumamoto, Japan). The absorbance was read using a fluorometer (CytoFluor; Applied Biosystems, Foster City, CA, USA) at 450 nm with a reference wavelength of 630 nm.

2.7. Apoptosis measurements

Drug-induced apoptosis following exposure to doxorubicin (Sigma) was detected in NHL cells. Then, apoptotic cells were detected using Annexin-V-FLUOS Staining Kit (Roche) according to the manufacture's protocol. Cells were washed three times and resuspended in 100L of AnnexinV-FLUOS labeling solution at a concentration of 1×10^6 cells/mL incubate in the dark for 15 min. Flow cytometry (BD FACSAriaII) was performed to analyze the labeled cells.

2.8. Soft agar colony assays

In short, cells were suspended at 1×10^3 cells in 0.5 mL of 0.35% agar solution (Sigma–Aldrich, St. Louis, MO, USA) containing RPMI 1640 cell culture medium and layered on top of a 0.8% agar layer in 24-well plates. Plates were then maintained for 14 days at 37 °C with 5% CO2. Cell colonies were stained with 0.5% crystal violet and visualized by microscopy.