Int J Clin Exp Pathol 2016;9(9):9356-9363
www.ijcep.com /ISSN:1936-2625/IJCEP0033100

Original Article

Expression of histone deacetylases 1 in newly diagnosed diffuse large B-cell lymphoma and its prognostic significance

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Received June 1, 2016; Accepted July 9, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: The expression pattern, clinical significance and prognostic value of the histone deacetylase 1 (HDAC1) were investigated in a cohort (n=89) of newly diagnosed diffuse large B-cell lymphoma (DLBCL) patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), through immunohistochemical (IHC) analysis in this study. High HDAC1 expression was detected in 88.89% (80/89) cases and was observed more frequently in non-germinal center B-cell (non-GCB) type compared with GCB type but without statistical significance (95.65% versus 83.72%, *P*=0.083). In addition, we found that high HDAC1 expression was significantly related to high National Comprehensive Cancer Network (NCCN)-International Prognostic Index (IPI) score (*P*=0.024). For cases of low HDAC1 expression, the 5-year overall survival (OS) was 100%, which was significantly higher than cases of high HDAC1 expression (36.2%, *P*=0.002). The 5-year progression-free survival (PFS) for low and high HDAC1 expression group was 77.8% and 31.3%, respectively, with statistical significance (*P*=0.021). Our findings supported the strategy of targeting HDAC1 in DLBCL patients, and moreover, proved the prognostic value of HDAC1 in DLBCL treatment.

Keywords: Histone deacetylases 1, diffuse large B-cell lymphoma, immunohistochemistry, epigenetics

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHL), accounting for approximately 30% of all lymphomas [1]. In the rituximab era, more than half of DLBCL patients can be cured with standard immunochemotherapy regimen consisting of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), however approximately 30~40% of patients eventually relapse or become refractory that remains a major cause of morbidity and mortality due to the limited therapeutic options [2]. DLBCL patients usually present with variable clinical, histological, immunophenotypic and cytogenetic features, thus the disease is a heterogeneous disease, which could partially explain the failure treatment of some patients [3]. Gene expression profiling (GEP) and genome sequencing analyses have increased our understanding of DLBCL subtypes and molecular basis of chemotherapy resistance [3-6]. In this regard, DLBCL can be stratified into two subtypes that transcriptionally resemble normal germinal center B-cells (GCB) or post-GCB activated B cells (ABC) [4]. These two subtypes have different genetic mutation landscapes, pathobiology, and outcomes following R-CHOP immunochemotherapy [4]. However, it should be noted that there is still obvious heterogeneity even when ABC and GCB are considered as separate diseases [7, 8].

Inactivation of tumor suppressor genes due to epigenetic alterations including histone modification, methylation, and small silencing ribonucleic acid (RNA), contributes to the development of cancer in a variety of tissues [9]. It is well known that the balance of acetylation and deacetylation is finely regulated through the activity of two opposing classes of enzymes, the histone/protein lysine acetyltransferases (HAT) and histone deacetylases (HDAC). Mam-

malian class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8) are predominantly expressed in the nucleus and are major mediators of histone deacetylation, whereas the class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) and class IIb HDACs (HDAC6 and HDAC10) are mainly expressed in the cytoplasm and exert deacetylation of non-histone proteins [10]. Previous studies suggest that the transcriptional repression of tumor-suppressor genes by overexpression and aberrant recruitment of HDACs to their promoter is a common phenomenon in tumorigenesis and progression [11]. For example, overexpression of class I HDACs (HDAC1, HDAC2 and HDAC3) has been found in a variety of tumors such as colon and breast carcinomas [11-14]. Also, class I HDACs (HDAC1, HDAC2 and HDAC3) and HDAC6 have been reported to have increased expression in DLBCL [11, 14]. Recently, Min *et al*. showed that HDAC1 expression (intensities of 2+ and 3+) was found in the majority of DLBCL, which is significantly correlated with a poorer survival [15]. However, Lee *et al*. reported only 12 (13.2%) cases expressed HDAC1 with faint staining intensity [16]. Based on the above background, in this study we evaluated the expression of HDAC1 in DLBCL through immunohistochemistry (IHC), and analyzed whether expression of HDAC1 influences survival of DLBCL patients treated with immunochemotherapy.

Patients and methods

Patients

A cohort of 89 patients with newly diagnosed DLBCL derived from the Department of Hematology, the First Affiliated Hospital, Zhejiang University College of Medicine, who received the R-CHOP-based chemotherapy regimen as first-line chemotherapy. All cases were carefully reviewed by hematopathologists and diagnosed according to the World Health Organization (WHO) classification criteria [1]. Patients were excluded if they had any of the following: DLBCL coexistent with or transformed from low-grade B-cell lymphoma, intravascular large B-cell lymphoma, DLBCL associated with chronic inflammation, primary mediastinal large B-cell lymphoma, primary cutaneous B-cell lymphoma, primary central nervous system DLBCL, T-cell or histiocyte-rich large B-cell lymphoma, anaplastic lymphoma kinase (ALK)-positive DLBCL, Epstein-Barr virus (EBV) positive DLBCL of elderly, human immunodeficiency virus related DLBCL, and DLBCL with grey zone features. The study protocols were approved by Ethics Committee of the First Affiliated Hospital, Zhejiang University College of Medicine.

Clinical data, including age, gender, sites of involvement, B symptoms, serum lactate dehydrogenase (LDH) concentrations and Eastern Cooperative Oncology Group (ECOG) performance status (PS), were obtained from the archive of the pathology department and the medical record of the hematology department. The cases were staged according to the Ann Arbor staging system, and enhanced National Comprehensive Cancer Network (NCCN)-International Prognostic Index (IPI) risk factors were also evaluated [17]. Complete remission (CR) or unconfirmed (CRu), and partial remission (PR) were evaluated according to the International Working Group (IWG) response criteria for NHL [18]. Overall survival (OS) was defined from the date of diagnosis to the date of last follow-up or death. Moreover, progression-free survival (PFS) was defined from the date of diagnosis to the date of progression, second tumor or death.

Histopathologic and IHC assessment

All tissue specimens from the patients were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Then paraffin sections were stained with hematoxylin-eosin (H&E) for microscopic examination. The sections were pretreated with microwave irradiation for antigen retrieval. To block the endogenous peroxidase activity, slides were incubated with 3% H_2O_2. IHC was performed using the following primary antibodies: cluster of differentiation (CD) 20, CD10, B-cell lymphoma-2 (BCL2), B-cell lymphoma-6 (BCL6), Ki-67, multiple myeloma oncogene 1 (MUM1) and MYC (Abcam, Cambridge, Britain, 1:100). GCB and non-GCB types were determined by Hans method [19]. In addition, the investigational IHC analyses for HDAC1 (Santa Cruz Biotechnology, CA, USA, 1:100) were performed. All the procedures were conducted in accordance with the manufacturer's instructions. The HDAC1 staining intensity was scored from 0 to 3, representing lack of staining (0, if 0-5%), mild staining (1, if 6-33%), intermediate staining (2, if 34-66%)

Table 1. Clinical characteristics of 89 DLBCL patients with regard to HDAC1 expression

Patients characteristics	HDAC1 High expression N (%)	HDAC1 Low expression N (%)	*P* Value
Patients	80 (88.89)	9 (11.11)	
Gender			
Male	52 (65.00)	5 (55.56)	
Female	28 (35.00)	4 (44.44)	0.717
Age, y			
60 or younger	47 (58.75)	8 (88.89)	
Older than 60	33 (41.25)	1 (11.11)	0.145
B symptoms			
Absent	58 (67.65)	9 (100.0)	
Present	22 (32.35)	0 (0)	0.105
ECOG			
0~1	44 (55.00)	8 (88.89)	
2~4	36 (45.00)	1 (11.11)	0.074
Stage			
I/II	35 (43.75)	7 (77.78)	
III/IV	45 (56.25)	2 (22.22)	0.078
Extranodal site*			
Absent	43 (53.75)	7 (77.78)	
Present	37 (46.25)	2 (22.22)	0.289
LDH			
Normal	58 (72.50)	8 (88.89)	
Elevated	22 (27.50)	1 (11.11)	0.437
BCL-6			
Negative	31 (38.75)	5 (55.56)	
Positive	49 (61.25)	4 (44.44)	0.476
Subtype			
GCB	36 (45.00)	7 (77.78)	
Non-GCB	44 (55.00)	2 (22.22)	0.083
NCCN IPI			
0~3	48 (60.00)	9 (100.0)	
4~8	32 (40.00)	0 (0)	0.024
Response			
CR/CRu/PR	69 (86.25)	9 (100.0)	
No Response	11 (13.75)	0 (0)	0.594

*Disease in bone marrow, CNS, liver, gastrointestinal tract, or lung.

and strong staining (3, if 66-100%). Tumors with a score 0-1 were interpreted as low expression, which tumor samples with a score 2-3 were considered as high expression.

Statistical analysis

OS and PFS were analyzed by Kaplan-Meier method, with difference compared by the log-rank test. Chi-square test was used to examine the relationships between HDAC1 and clinical characteristics. Fisher exact test were used to analysis the correlation between the variables. A *P* value less than 0.05 was considered statistically significant. SPSS Statistics V22 (IBM) was used for statistical analyses.

Results

Clinicopathologic features

The detailed clinical and pathological data of all patients with DLBCL are summarized in **Table 1**. Of the 89 cases, 57 (64%) were man and 32 (36%) were woman (male to female ratio, 1.78:1). The patients ranged in age from 15 to 81 years (median age 57 years). B-symptoms were present in 24.72% of patients. 52.81% patients had advanced Ann Arbor stage disease (Stage III or IV), and extranodal sites were involved in 43.82% patients. Under the classification according to Hans algorithm (19), 46 cases (51.69%) are non-GCB and 43 (48.31%) are GCB type.

Relationships between HDAC1 expression and clinicopathologic variables

Consistent with a previous report, HDAC1 was stained in the nucleus of tumor cells with variable intensities and proportions in DLBCLs (**Figure 1**). The corrections between HDAC1 and clinicopathologic features are shown in **Table 1**. A total of 80 and 9 cases were subclassified into high and low HDAC1-expression groups, respectively. High expression-HDAC1 was more frequently observed in the patients with NCCN-IPI scores ≥4 than the cases with NCCN-IPI scores ≤3 (*P*=0.024). HDAC1 positivity was observed more often in the non-GCB (95.65%) than in the GCB subtype (83.72%), but this difference was not significant (*P*=0.083). Furthermore, there were also no significant correlations between expression of HDAC1 and other clinical features such as age,

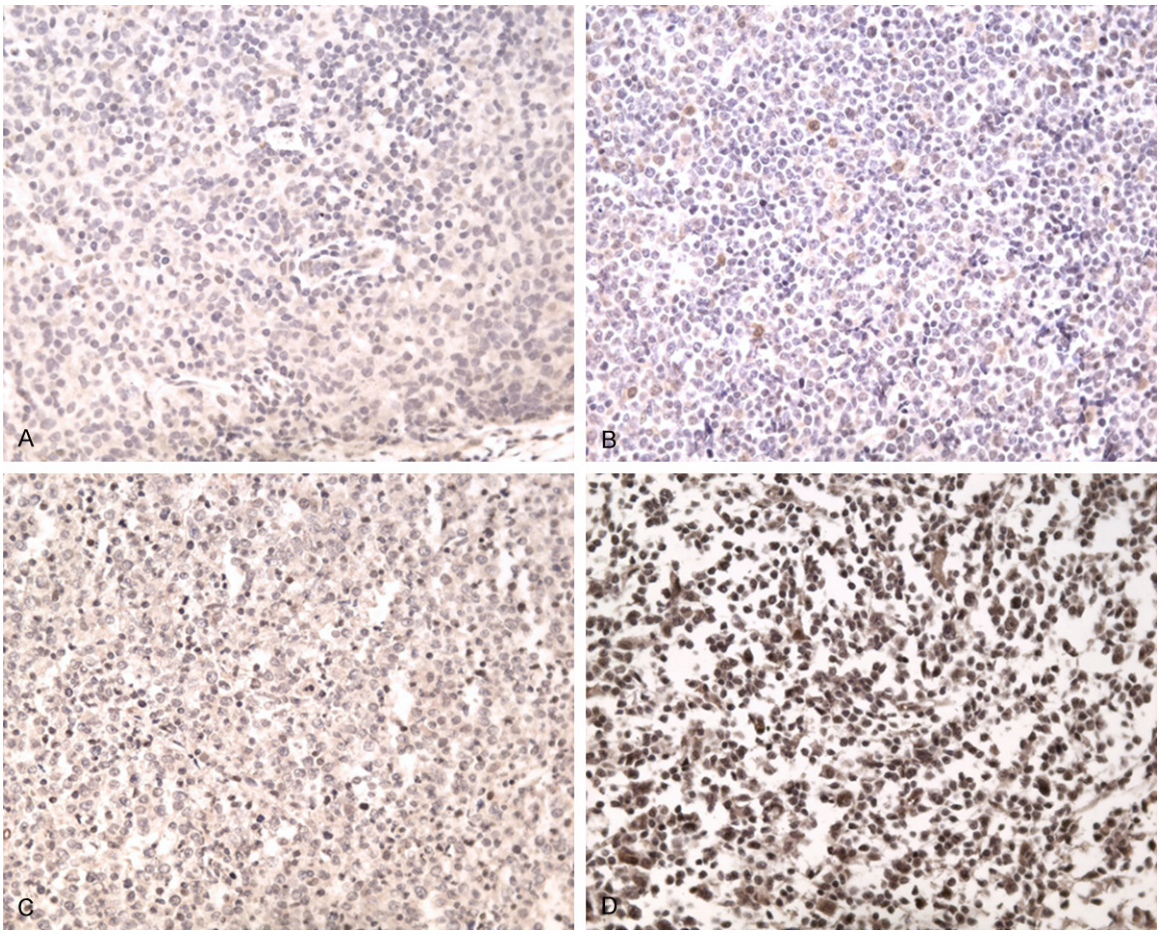

Figure 1. The intensity of HDAC1 expression in DLBCL. (A) lack of staining, score 0, (B) mild staining score 1, (C) intermediate staining, score 2, and (D) strong staining, score 3.

gender, B-symptoms, PS, Ann Arbor stage, extranodal infiltrations, and LDH level.

Association of clinical outcome with HDAC1 expression levels

Therapy response including CR, CRu, and PR was achieved in 78 (87.6%) patients. We noticed no significant difference in the likelihood of achieving therapy response regarding HDAC1 immunoexpression (P=0.594). By the time of closeout, with a median follow-up of 45 months, for all the 89 patients, the estimated 5-year OS rate was 42.7%, and the estimated PFS at 5-year was 36.0% (**Figure 2A** and **2B**). When the cases were categorized into GCB and non-GCB types on Hans algorithm, patients with GCB had better OS (P=0.016) and PFS (P=0.013) than those with non-GCB type (**Figure 2C** and **2D**). To elucidate the subgroups in which HDAC1 could predict the prognosis, we next analyzed the association between HDAC1 expression levels and clinic outcome (**Figure 2E** and **2F**). The results showed that in cases with high HDAC1-expression, the 5-year OS was significantly shorter than that with low expression (36.2% vs. 100%, P=0.002). The 5-year PFS was also significantly shorter than that with low HDAC1-expression group (31.3% vs. 77.8%, P=0.021).

Discussion

Because HDACs modulate a variety of cellular functions that are involved in tumorigenesis, cell growth, survival, and homologous recombination, they are considered a promising target for cancer therapy [9, 20]. A recent study using combined genetic and pharmacological approaches showed that knockdown of HDAC3 in Eμ-*Myc* lymphoma resulted in a block of proliferation, which was not prevented by Bcl-2 over-

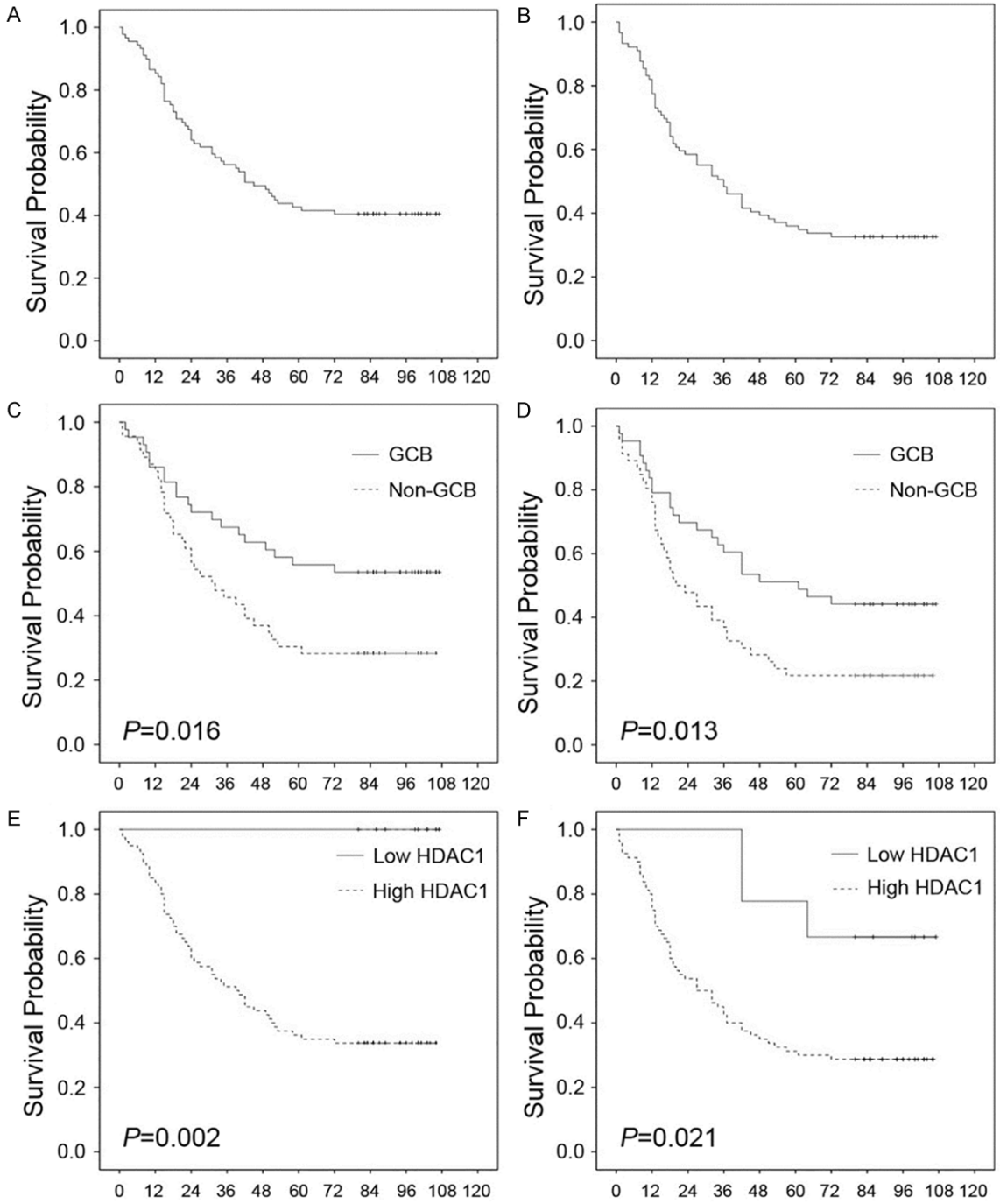

Figure 2. Survival data in DLBCL. A and B. The OS and PFS for all the patients respectively. C and D. Survival impact of GCB and non-GCB subtype in DLBCL, the subtype of DLBCL was correlated with different outcome in OS and PFS. E and F. Survival impact of HDAC1 expression in DLBCL, the higher HDAC1 expression was significantly related to poorer outcome in both OS and PFS.

expression and caspase inhibition [21]. In addition, genetic codepletion of HDAC1 with HDAC2 was pro-apoptotic in Eμ-*Myc* lymphoma *in vitro* and *in vivo* [21]. This study strongly supports the notion that class I HDACs (HDAC1, HDAC2 and HDAC3) play an important role in the proliferation of lymphoma cells. Distinct HDACs are found overexpressed in a variety of lymphomas including DLBCL, follicular lymphoma, and chronic lymphocytic leukemia [22]. Marquard L

et al. examined immunohistochemical expression of HDAC1, HDAC2 and HDAC6 in 31 DLBCL and reported that all three markers shows high expression in DLBCL compared with normal lymphoid tissue [14]. Consistent with this report, another study showed that tumors from 70 DLBCL patients (89.7%) were positive for HDAC1 [15]. In contrast, there was a study that showing that HDAC1 was expressed in 13.2% cases with faint staining intensity [16]. These seemingly conflicting results could explain the heterogeneity of lymphoma. However, recent *in vitro* evidence suggests that selective inhibition of HDAC1 and HDAC2 activity by a small molecule drug causes cytotoxic effect in enhancer of zeste homologue 2 (EZH2) gain-of-function mutant DLBCL cells [23]. Taken together, these data suggest that HDAC1 is considered to be a promising therapeutic target in DLBCL.

In this study, we found that HDAC1 was expressed at high levels in 88.89% (80/89) of DLBCL patients. Prior data indicated that non-GCB DLBCL patients have significantly worse PFS and OS [24, 25]. Consistent with these results, we also observed that GCB group had a better OS and PFS than the non-GCB group. There is a trend that high expression HDAC1 in non-GCB group is more frequently than that in GCB group, although without significance. Recently, it was demonstrated that the NCCN-IPI is more powerful than the IPI for predicting survival in the immunochemotherapy era [17]. In present study, when stratifying out patients into two groups according to NCCN-IPI (low and low-intermediate-risk versus high-intermediate and high-risk), high-expression HDAC1 was observed in the cases with high-intermediate and high-risk (32/80; 40%), but not in the cases with low and low-intermediate-risk (P=0.024). Interestingly, our results showed that the immunohistochemical expression of HDAC1 was associated with poor clinical outcome of DLBCL patients. To our knowledge, this is the first report showing that high expression levels of HDAC1 are associated with shorter survival time in DLBCL.

In summary, we show here that the majority of patients with DLBCL overexpress HDAC1 and that HDAC1 is a new immunohistochemical prognostic marker. Our findings suggest that the HDAC1 may be a potential therapeutic target for the treatment of DLBCL.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81200384, 81500110 and 81302036), Funds of Health and Family Planning Commission of Zhejiang Province (No. 2014RCA006).

Disclosure of conflict of interest

None.

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