Int J Clin Exp Pathol 2018;11(9):4472-4482
www.ijcep.com /ISSN:1936-2625/IJCEP0079320

Original Article

Expression of hypoxia-inducible factor-1a predicts benefit from rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in diffuse large B-cell lymphoma

Jie Jin[1,2*], Lihong Cao[1,2,3*], Lijun Wang[4], Zhaoming Wang[5], Wei Ding[5], Liping Mao[1,2], Shanshan Suo[1,2], Zhengping Zhuang[6], Hongyan Tong[1,2]

[1]Department of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, People's Republic of China; [2]Institute of Hematology, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [3]Department of Hematology, Shulan (Hangzhou) Hospital, Hangzhou, People's Republic of China; [4]Department of Pathology, The Fourth Affiliated Hospital of Zhejiang University, Yiwu 322000, People's Republic of China; [5]Department of Pathology, The First Affiliated Hospital of Zhejiang University, Hangzhou, People's Republic of China; [6]Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA. [*]Equal contributors.

Received May 9, 2018; Accepted June 21, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: Hypoxia-inducible factor-1α (HIF-1α) has been identified as an unfavorable prognostic factor in most solid tumors. However, HIF-1α was suggested to predict improved survival in Western patients with diffuse large B-cell lymphoma (DLBCL) under rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) treatment. We studied HIF-1α protein expression by immunohistochemical staining of 155 paraffin-embedded specimens from Chinese patients with DLBCL treated with R-CHOP or CHOP. Results were correlated with patient outcome. HIF-1α expression had no impact on survival for the patients treated with CHOP. In the R-CHOP-treated group, however, HIF-1α expression was significantly correlated with superior OS and EFS (P = 0.048 and 0.040, respectively). Moreover, HIF-1α expression maintained independent prognostic value for OS (RR, 0.41; 95% CI, 0.19-0.92; P = 0.030) and EFS (RR, 0.53; 95% CI, 0.31-0.90; P = 0.020) when it was adjusted by IPI stratification. Therefore, HIF-1α expression benefits from R-CHOP in DLBCL.

Keywords: Diffuse large B-cell lymphoma, immunohistochemistry, hypoxia-inducible factor, prognosis

Introduction

Hypoxia-inducible factor (HIF)-1 is a heterodimer consisting of an oxygen-sensitive HIF-1α subunit and a constitutive expressed HIF-1β subunit [1, 2]. HIF-1α is a pivotal transcriptional factor, controlling the expression of more than 200 hypoxic stress-related genes, which regulate a wide range of cellular processes including angiogenesis, erythropoiesis, and glycolysis to maintain O_2 homeostasis [3-5]. Hypoxia is a common characteristic of many solid tumors [6]. HIF-1α can be activated in tumors under normoxic conditions, as well as hypoxia [7]. HIF-1α participates in multiple aspects of tumorigenesis, including differentiation, proliferation, and metastasis [8]. High HIF-1α expression has been suggested to be associated with poor prognosis in most solid tumors [9-18]. However, HIF-1α expression has also been shown to be predictive of improved survival in some other solid tumors such as invasive bladder cancer [19], head and neck squamous cell carcinoma [20], and diffuse large B-cell lymphoma (DLBCL) [21, 22].

DLBCL is a biologically and clinically heterogeneous group of lymphomas [23]. Currently, approximately 40% of patients with DLBCL experience early treatment failure or eventual relapse with standard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) [24]. For the past few years, the prognostic significance of MYC, BCL2, BCL6, and

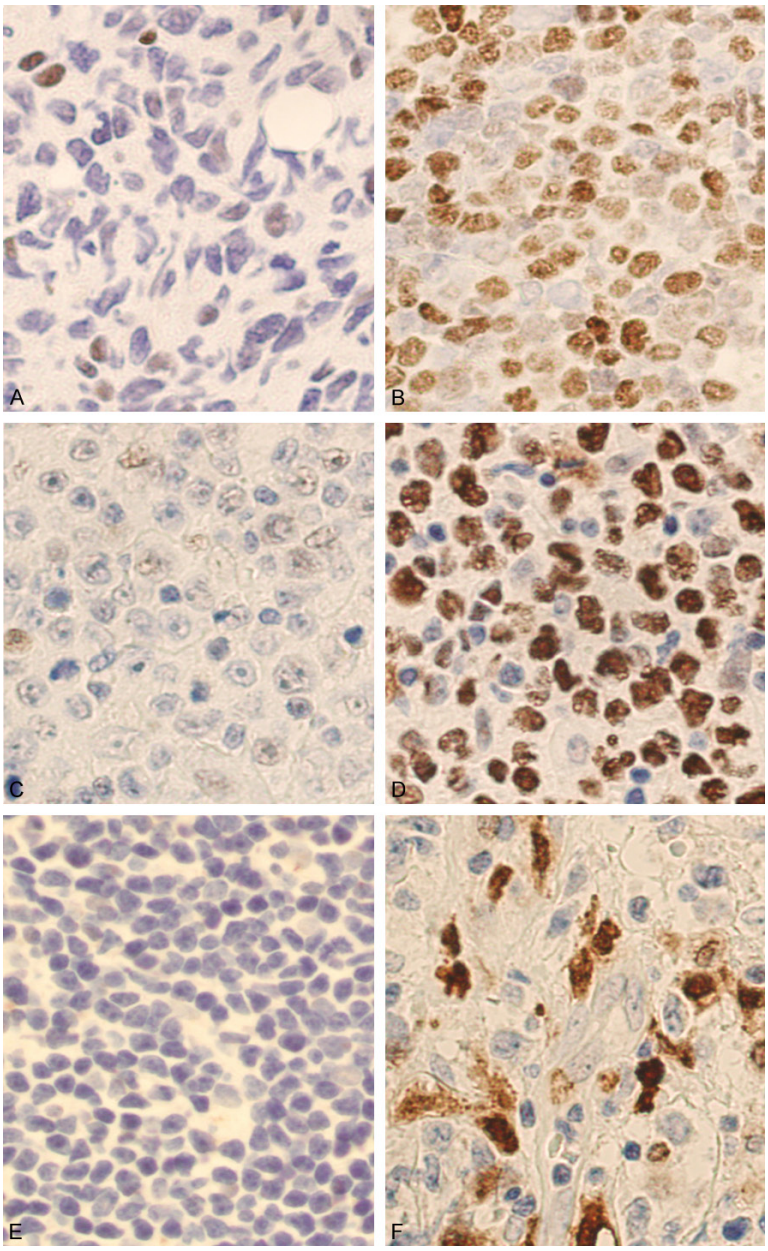

Figure 1. Immunohistochemical staining showing negativity for (A) MYC, (C) p53, (E) HIF-1α and positivity for (B) MYC, (D) p53, (F) HIF-1α in diffuse large B-cell lymphomas. All cases are shown at ×40 magnification. All staining patterns are distinctly nuclear.

especially their combination has received considerable attention in the context of DLBCL, particularly treated with R-CHOP [25-29]. HIF-1α, as another biological marker, has also been evaluated [21, 22]. Unfortunately, the two studies were both based on Western population. In fact, DLBCL in China appears to differ from those in Western countries. Seventy-nine percent of Chinese DLBCL are categorized into Non-GCB subtype relative to 42% of Western DLBCL according to Hans algorithm [30, 31]. Therefore, we developed this study to explore the prognostic impact of HIF-1α expression in Chinese patients with DLBCL treated with R-CHOP.

Materials and methods

We searched the Electronic Medical Record in the First Affiliated Hospital of Zhejiang University School of Medicine to establish a DLBCL cohort. A tissue diagnosis of DLBCL was confirmed by central review, based on the 2008 World Health Organization classification. Any tumor with any confirmed follicular architecture was rule out. We also excluded transformed lymphomas, HIV-associated lymphomas and post-transplant lymphomas. All the included cases received standard R-CHOP/CHOP, with sufficient clinical data and adequate formalin-fixed, paraffin-embedded (FFPE) tissues. Finally, a total of 155 de novo diagnosed patients with DLBCL between June 2010 and May 2015 were eligible for the study. This study was approved by the institutional review boards of the First Affiliated Hospital of Zhejiang University School of Medicine.

Immunohistochemistry

Sections were subjected to standard staining protocols. Immunohistochemistry with antibodies against CD10, BCL6, MUM1, and Ki67 had been performed at the time of diagnosis. Immunohistochemistry with antibodies against MYC, p53 and HIF-1α were done on FFPE sections of 4 μm, placed on adhesive-coated slides. Antigen retrieval was achieved by heat-induced epitope retrieval methods in a steamer. The slides were incubated with primary monoclonal antibodies to MYC (Clone Y69; Abcam, Cambridge, USA;

Table 1. Patient clinical characteristics based on HIF-1α expression

Characteristic	HIF-1α$^+$ (n = 101)	HIF-1α$^-$ (n = 52)	P
	N (%)	N (%)	
Age, y			
Median (Range)	55 (19-78)	57 (17-72)	0.341
≤60	75 (74.3)	35 (67.3)	0.365
>60	26 (25.7)	17 (32.7)	
Stage			0.536
I-II	28 (27.7)	12 (23.1)	
III-IV	73 (72.3)	40 (76.9)	
ECOG PS			0.001
0-1	73 (72.3)	24 (46.2)	
≥2	28 (27.7)	28 (53.8)	
LDH			0.062
Normal	51 (50.5)	18 (34.6)	
Elevated	50 (49.5)	34 (65.4)	
Extranodal sites			0.992
0-1	70 (69.3)	36 (69.2)	
≥2	31 (30.7)	16 (30.8)	
IPI score			0.002
0-2	65 (64.4)	20 (38.5)	
3-5	36 (35.6)	32 (61.5)	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; HIF, hypoxia-inducible factor; IPI, International Prognostic Index; LDH, lactate dehydrogenase; PS, performance status.

dilution 1:100), p53 (DO-7; Abcam, Shanghai, China; dilution 1:150) and HIF-1α (EP1215Y; Abcam, Shanghai, China; dilution 1:100) in serum albumin at 37±1°C for 60 min. Detection was accomplished with a universal immuno-peroxidase polymer (PV-8000, Anti-Mouse/Rabbit Immunohistochemical Staining Reagent).

Two experienced hematopathologists evaluated all the immunohistochemical staining semiquantitatively in 10% increments irrespective of staining intensity, without knowledge of patient outcome. Any disagreements were resolved by joint review over a multi-headed microscope. The cutoff values of MYC and p53 were 40% [26-28] and 30% [32], respectively. HIF-1α was judged as positive if any definitive staining of lymphoma cells was believed positive [21]. For statistical convenience, a superscript signal "+" represented a biomarker expression of ≥ the cut-point and "-" showed a biomarker expression of < the cutoff. **Figure 1** presented the immunohistochemical staining of MYC, p53 and HIF-1α.

Statistical analysis

Subgroups according to patient characteristics by HIF-1α status were compared using X^2-test and, if necessary, Fisher's exact test. X^2-test was also used to evaluate the correlation between HIF-1α and other biomarker expression. EFS was defined as the time from diagnosis to disease progression, start of salvage treatment, additional (unplanned) treatments, relapse, or death from any cause. Patients without any incident above at last contact were treated as censored data for EFS analysis. OS was defined as the time from diagnosis to death of any cause. Patients who were alive at last contact, were treated as censored data for OS analysis. EFS and OS were estimated by Kaplan-Meier method. In univariate survival analyses, log-rank tests were performed. In multivariate survival analysis, Cox Proportional Hazard Model was used. Relative risks (RR) with 95% confidence intervals (CIs) and *P* values were presented. All *P* values were based on 2-sided tests. The significance level was 0.05. Statistical analyses were conducted with IBM SPSS Statistics 20.0.

Results

HIF-1α expression

Of the included 155 cases with DLBCL, two were excluded because their staining for HIF-1α was not interpretable, leaving 153 in the final analysis. Of patients who had elevated HIF-1α protein expression (101 of 153), 80.1% (81 of 101) had higher (>10% malignant cells) HIF-1α staining. HIF-1α was expressed in 66.3% (65/98) of patients treated with R-CHOP relative to 65.5% (36/55) treated with CHOP (P = 0.913). We also determined HIF-1α expression status according to cell of origin (COO), on the basis of the Hans algorithm [33]. HIF-1α was seen in 72.4% (42/58) of GCB subgroup compared with 62.1% (59/95) of Non-GCB subgroup (P = 0.913).

As provided in **Table 1**, the HIF-1α$^+$ patients had similar percentage of stage III-IV and at least two extranodal sites involved compared with those with HIF-1α$^-$ ones. The patients with HIF-1α$^+$ were younger and had a lower proportion of elevated lactate dehydrogenase (LDH) relative to those with HIF-1α$^-$ (P = not significant). However, the HIF-1α$^+$ patients had significantly decreased incidence of worse personal status (PS) and IPI score of 3-5 compared with the

Table 2. Patient clinical characteristics based on treatment

Characteristic	R-CHOP (n = 98)	CHOP (n = 55)	P
	N (%)	N (%)	
Age, y			
Median (Range)	57 (17-78)	52 (18-73)	0.789
≤60	70 (71.4)	40 (72.7)	0.864
>60	28 (28.6)	15 (27.3)	
Stage			0.031
I-II	20 (20.4)	20 (36.4)	
III-IV	78 (79.6)	35 (63.6)	
ECOG PS			0.073
0-1	57 (58.2)	40 (72.7)	
≥2	41 (41.8)	15 (27.3)	
LDH			0.279
Normal	41 (41.8)	28 (50.9)	
Elevated	57 (58.2)	27 (49.1)	
Extranodal sites			0.031
0-1	62 (63.3)	44 (80)	
≥2	36 (36.7)	11 (20)	
IPI score			0.065
0-2	49 (50.0)	36 (65.5)	
3-5	49 (50.0)	19 (34.5)	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative. Oncology Group; HIF, hypoxia-inducible factor; IPI, International Prognostic Index; LDH, lactate dehydrogenase; PS, performance status.

HIF-1α$^-$ patients (P<0.05). Therefore, the HIF-1α$^+$ patients were less serious than the HIF-1α$^-$ ones.

As presented in **Table 2**, the patients treated with R-CHOP had similar age and LDH distribution to those treated with CHOP (P = not significant). However, the R-CHOP subgroup probably had worse PS relative to the CHOP subgroup (P = 0.073). Moreover, the R-CHOP-treated patients had a significantly larger percentage of stage III-IV and at least two extranodal sites involved compared with the CHOP-treated ones (P<0.05). So it was reasonable that IPI score in the R-CHOP subgroup was probably higher than that in the CHOP subgroup (P = 0.065). Therefore, the condition in the R-CHOP subgroup was probably more serious compared with that in the CHOP subgroup.

HIF-1α compared with other protein biomarkers in the R-CHOP-treated group

Among patients treated with R-CHOP, we detected whether HIF-1α status was associated with the expression of other protein biomarkers. As presented in **Table 3**, HIF-1α status had no association with the expression of BCL6, CD10, MUM1, Ki-67, BCL2, or MYC expression. However, it was detected that HIF-1α expression was marginally negatively associated with p53 expression (P = 0.060).

Table 3. The expression of HIF-1α compared with the expression of other biomarkers in the R-CHOP- treated group

Biomarker	HIF-1α$^+$	HIF-1α$^-$	P
	N (%)	N (%)	
BCL6			
Negative	18 (27.7)	7 (21.2)	0.487
Positive	47 (72.3)	26 (78.8)	
CD10			
Negative	50 (76.9)	25 (75.8)	0.898
Positive	15 (23.1)	8 (24.2)	
MUM1			
Negative	20 (30.8)	10 (30.3)	0.962
Positive	45 (69.2)	23 (69.7)	
Ki-67			
≥75%	45 (69.2)	22 (66.7)	0.796
<75%	20 (30.8)	11 (33.3)	
BCL2			
Negative	21 (32.3)	7 (21.2)	0.251
Positive	44 (67.7)	26 (78.8)	
MYC			
Negative	48 (73.8)	20 (60.6)	0.179
Positive	17 (26.2)	13 (39.4)	
P53			
Negative	46 (70.8)	17 (51.5)	0.060
Positive	19 (29.2)	16 (48.5)	

HIF-1α expression and clinical outcome

In univariate analysis, first, we evaluated the association between HIF-1α expression and clinical outcome of all patients. With a median follow-up of 71.53 months (range, 41.93-104.37 months), there was significantly improved OS and EFS for the patients with HIF-1α$^+$ than those with HIF-1α$^-$ (P = 0.048 and 0.040, respectively, **Figure 2A** and **2B**). Subsequently we investigated the relationship between HIF-1α expression and survival by treatment groups. No difference was discovered in OS or EFS among the CHOP-treated group (**Figure 2C** and **2D**). In the R-CHOP-treated group, however, HIF-1α expression was significantly associated with superior OS and EFS (5-year OS: 81% versus 54%, P = 0.001,

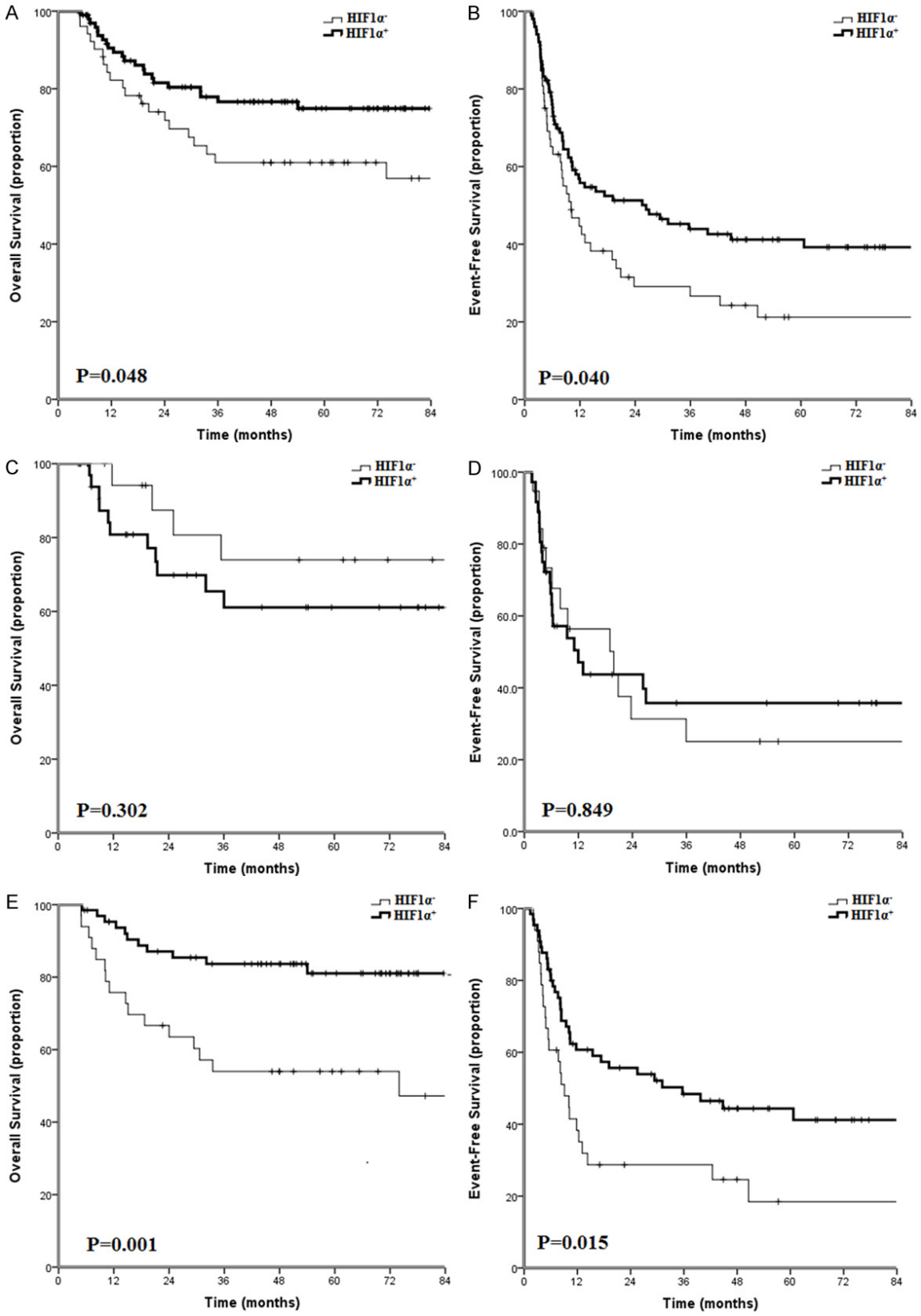

Figure 2. Overall survival and event-free survival according to HIF-1α$^+$ and HIF-1α$^-$ status. Kaplan-Meier curves of (A) OS and (B) EFS in 153 patients with diffuse large B-cell lymphoma (DLBCL) showed significantly improved OS and EFS for the patients with HIF-1α$^+$ than those with HIF-1α$^-$ (P = 0.048 and 0.040, respectively). Kaplan-Meier

curves of (C) OS and (D) EFS in 52 patients with DLBCL treated with cyclophosphamide, doxorubicin, oncovin, and prednisone (CHOP) grouped on the basis of HIF-1α protein expression showed no correlation with HIF-1α protein expression. Kaplan-Meier curves of (E) OS and (F) EFS in 101 patients with DLBCL grouped on the basis of HIF showed HIF-1α expression was significantly associated with superior OS and EFS (P = 0.001 and 0.015, respectively).

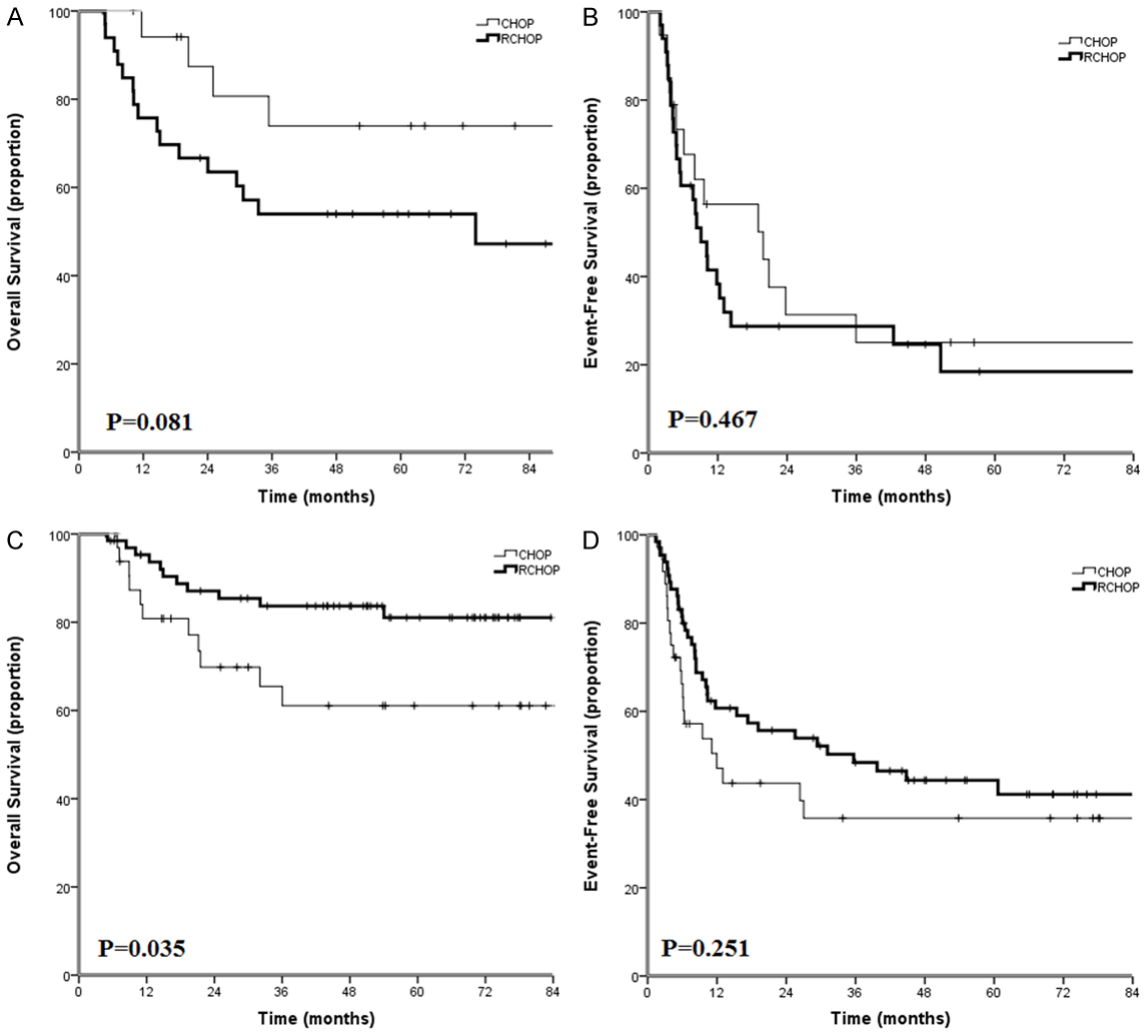

Figure 3. Overall survival and event-free survival according to treatment with R-CHOP and CHOP. Kaplan-Meier curves of (A) OS and (B) EFS in patients with HIF-1α$^-$ showed no correlation with treatment. Kaplan-Meier curves of (C) OS in patients with HIF-1α$^+$ showed a better OS among the patients treated with R-CHOP than those with CHOP (P = 0.035). However, Kaplan-Meier curves of (D) EFS did not show an association between EFS and treatment in HIF-1α$^+$ subgroup.

Figure 2E; 5-year EFS: 40% versus 17%; P = 0.015; **Figure 2F**).

Then, we analyzed survival according to treatment with R-CHOP and CHOP by HIF-1α expression status. In the HIF-1α$^-$ subgroup, OS or EFS did not appear to be influenced by treatment (**Figure 3A** and **3B**). In contrast, in the HIF-1α$^+$ subgroup, a better OS was observed among the patients treated with R-CHOP than those with CHOP (P = 0.035, **Figure 3C**). However, we did not see an association between EFS and treatment (P = 0.251, **Figure 3D**) possibly because of small size in HIF-1α$^+$ subgroup treated with CHOP.

Next, we investigated whether the prognostic value of the HIF-1α status was dependent on COO in the R-CHOP-treated group. The HIF-1α$^+$ patients had significantly improved OS and EFS

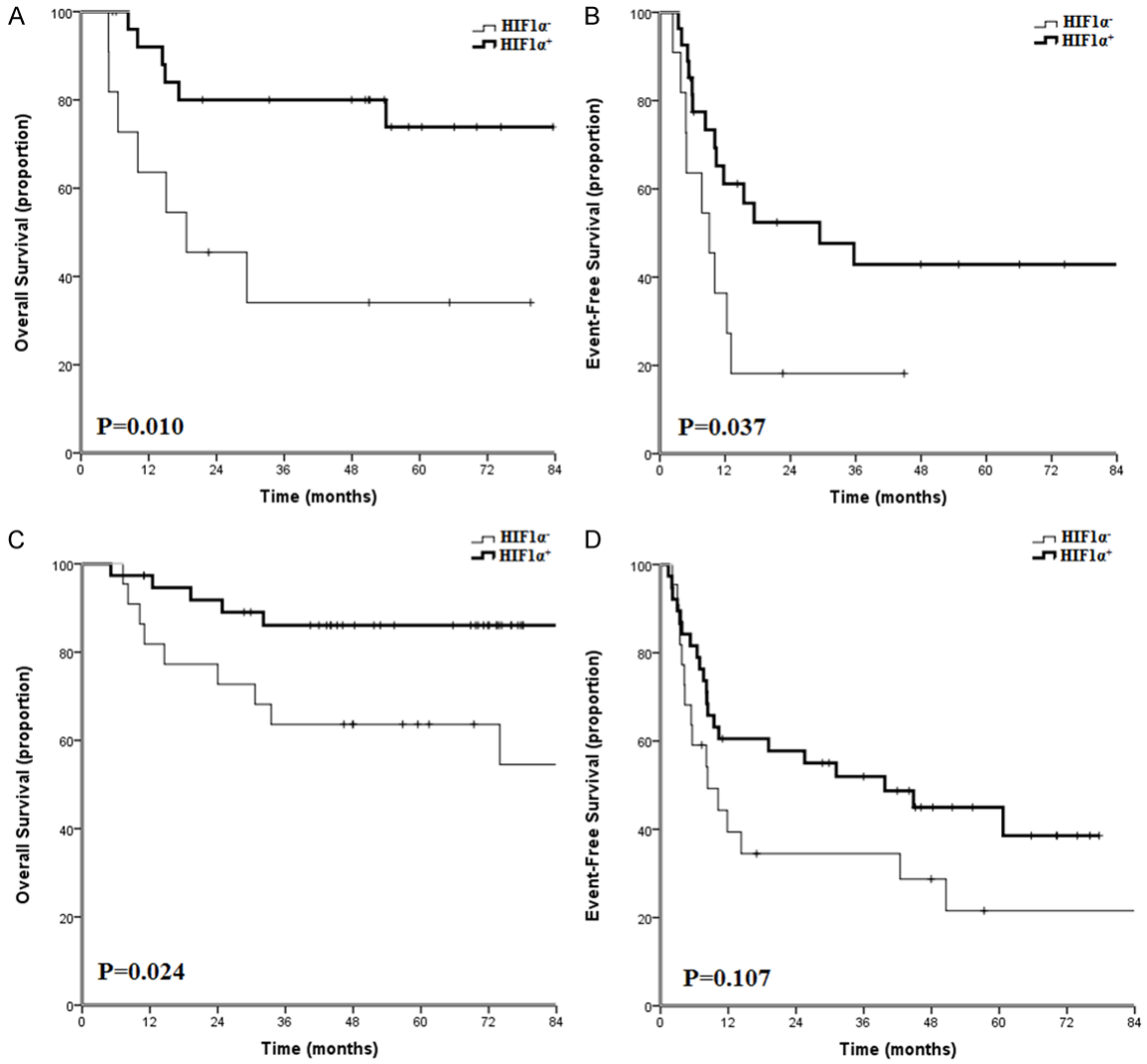

Figure 4. Overall survival and event-free survival according to HIF-1α status by COO in the R-CHOP treated group. Kaplan-Meier curves of (A) OS and (B) EFS in GCB subgroup showed that the HIF-1α$^+$ patients had significantly improved OS and EFS compared to the HIF-1α$^-$ ones (P = 0.010 and 0.037, respectively). Kaplan-Meier curves of (C) OS and (D) EFS in Non-GCB subgroup showed that HIF-1α expression was significantly associated with OS (P = 0.024) and marginally correlated with EFS (P = 0.107).

over the HIF-1α$^-$ ones within the GCB subgroups (P = 0.010 and 0.037, respectively, **Figure 4A** and **4B**). HIF-1α expression was significantly associated with OS in the non-GCB subgroup (P = 0.024, **Figure 4C**) and marginally correlated with EFS in the Non-GCB subgroup (P = 0.107, **Figure 4D**).

Finally, the prognostic significance of HIF-1α status was adjusted by IPI (0 to 2 and 3 to 5) in the R-CHOP-treated group. As a result, HIF-1α expression maintained independent prognostic value for OS (RR, 0.41; 95% CI, 0.19-0.92; P = 0.030) and EFS (RR, 0.53; 95% CI, 0.31-0.90; P = 0.020).

Discussion

In the present study, we found that high expression of HIF-1α protein is an independent favorable predictor of OS and EFS among patients with DLBCL treated with R-CHOP, consistent with prior reports [21, 22]. We also observed an association within both the GCB and the non-GCB subtypes of DLBCL. However, Evens et al [21] did not find a survival difference in the GCB subtype of DLBCL. Although the reason for the prognostic value of HIF-1α is unclear, some possible suggested reasons might explain it.

In spite of HIF-1α's pro-tumorigenic properties, HIF-1α has also been reported to negatively

regulate tumor growth. First, HIF-1α can indirectly induce cell cycle arrest by functionally counteracting MYC [34]. Second, HIF-1α is able to induce apoptosis. HIF-1α can induce the expression of the proapoptotic genes BNIP3 and NIX in a variety of human cancer cell lines. [35, 36]. Carmeliet et al [8] suggested that embryonic stem (ES) cells with HIF-1α$^+$ might be more prone to undergoing apoptosis under hypoxic conditions than ES cells with HIF-1α$^-$. Thirdly, HIF-1α over-expression is able to induce autophagy. HIF-1α can induce the transcription of BNIP3 by interacting with HIF response element (HRE). BNIP3 is a hypoxia-inducible member of the BCL2 superfamily of cell death regulators and plays a key role in regulating hypoxia-induced autophagy [37].

Despite vascular endothelial growth factor (VEGF)'s role in tumor angiogenesis, VEGF has also been reported to play another potential role in the context of hematolymphoid malignancies [38]. As an autocrine growth factor, VEGF acts on lymphoma cells directly through its receptors (VEGFRs). The combination of high VEGF and VEGFR1 expression has been found to predict improved survival in patients with DLBCL [39]. HIF-1α can activate the expression of VEGF [40]. It has been confirmed that there is a high concordance between HIF-1 expression and VEGF expression [41].

Reactive oxygen species (ROS) have been shown to directly lead to up-regulation of HIF-1α mRNA and HIF-1α protein levels [42, 43]. It has also been suggested that ROS is able to increase CD20 expression in the Burkitt lymphoma cell lines, Daudi and Raji [44]. Taken together, HIF-1α expression may reflect an increase in CD20 expression, making this subset of patients more likely to respond to rituximab.

Therefore, it is possible that HIF-1α expression is positively associated with clinical outcome in patients with DLBCL treated with R-CHOP. We did not demonstrate this correlation among the CHOP-treated patients probably due to the predominant role of rituximab, as in Evens et al.

In the present study, immunohistochemical analysis showed that HIF-1α expression had no association with COO as Evens et al and Powell et al observed. However, we demonstrated a trend that p53$^+$ was seen less commonly in the HIF-1α$^+$ subgroup compared with the HIF-1α$^-$ one while Evens et al and Powell et al did not investigate these trends. p53 has been demonstrated to predict poor outcome for R-CHOP-treated DLBCL patients [32]. Evens et al and Powell et al both detected a link between HIF-1α and BCL6 expression while our study did not disclose a link. The cause for these discrepancies may be that Chinese patients with DLBCL are different from Western ones.

There are two main limitations which should be considered when interpreting the data from the present study. First, the patients treated with R-CHOP accounted for two-thirds of DLBCL patients. Moreover, the condition in the R-CHOP subgroup was probably more serious compared with that in the CHOP subgroup. Therefore, there was a certain degree of selection bias in this study. Second, the sample in some subgroups was small and it is a retrospective study weakening its predictive value.

Nevertheless, high HIF-1α expression benefits from R-CHOP in Chinese DLBCL, as in Western DLBCL. These data suggest that there may be a significant interaction between HIF-1α activation and anti-CD20 monoclonal antibody treatment. A prospective study is needed to verify this correlation. It should be a focus whether and how rituximab targets HIF-1α expression in future studies.

Acknowledgements

We sincerely thank the other staffs in the department of pathology of the First Affiliated Hospital of Zhejiang University for their Job in helping through all the immunohistochemical staining. This study was funded by the Program for the Meaning of HIF-1α in Diffuse Large B-cell Lymphoma (Grant number: 2013KYA083). We wish to thank the Department of Pathology of the First Affiliated Hospital of Zhejiang University for the Immunohistology Study.

Disclosure of conflict of interest

None.

Address correspondence to: Hongyan Tong, Department of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310009, People's Republic of China. Tel: 86-571-87236628; Fax: 86-571-8723-6628; E-mail: zjuhongyantong@163.com

References

[1] Semenza GL. HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol 2001; 13: 167-71.

[2] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92: 5510-4.

[3] Wenger RH, Gassmann M. Oxygen(es) and the hypoxia-inducible factor-1. Biol Chem 1997; 378: 609-16.

[4] vens Semenza GL. Transcriptional regulation by hypoxia-inducible factor 1 molecular mechanisms of oxygen homeostasis. Trends Cardiovasc Med 1996; 6: 151-7.

[5] Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 1998; 12: 149-62.

[6] Dhani N, Fyles A, Hedley D, Milosevic M. The clinical significance of hypoxia in human cancers. Semin Nucl Med 2015; 45: 110-21.

[7] Semenza GL. Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. Crit Rev Biochem Mol Biol 2000; 35: 71-103.

[8] Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998; 394: 485-90.

[9] Yang SL, Liu LP, Jiang JX, Xiong ZF, He QJ, Wu C. The correlation of expression levels of HIF-1alpha and HIF-2alpha in hepatocellular carcinoma with capsular invasion, portal vein tumor thrombi and patients' clinical outcome. Jpn J Clin Oncol 2014; 44: 159-67.

[10] Wang Q, Hu DF, Rui Y, Jiang AB, Liu ZL, Huang LN. Prognosis value of HIF-1alpha expression in patients with non-small cell lung cancer. Gene 2014; 541: 69-74.

[11] Osada R, Horiuchi A, Kikuchi N, Yoshida J, Hayashi A, Ota M, Katsuyama Y, Melillo G, Konishi I. Expression of hypoxia-inducible factor 1alpha, hypoxia-inducible factor 2alpha, and von Hippel-Lindau protein in epithelial ovarian neoplasms and allelic loss of von Hippel-Lindau gene: nuclear expression of hypoxia-inducible factor 1alpha is an independent prognostic factor in ovarian carcinoma. Hum Pathol 2007; 38: 1310-20.

[12] Shibaji T, Nagao M, Ikeda N, Kanehiro H, Hisanaga M, Ko S, Fukumoto A, Nakajima Y. Prognostic significance of HIF-1 alpha overexpression in human pancreatic cancer. Anticancer Res 2003; 23: 4721-7.

[13] Hui EP, Chan AT, Pezzella F, Turley H, To KF, Poon TC, Zee B, Mo F, Teo PM, Huang DP, Gatter KC, Johnson PJ, Harris AL. Coexpression of hypoxia-inducible factors 1alpha and 2alpha, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival. Clin Cancer Res 2002; 8: 2595-604.

[14] Yoshimura H, Dhar DK, Kohno H, Kubota H, Fujii T, Ueda S, Kinugasa S, Tachibana M, Nagasue N. Prognostic impact of hypoxia-inducible factors 1alpha and 2alpha in colorectal cancer patients: correlation with tumor angiogenesis and cyclooxygenase-2 expression. Clin Cancer Res 2004; 10: 8554-60.

[15] Schindl M, Schoppmann SF, Samonigg H, Hausmaninger H, Kwasny W, Gnant M, Jakesz R, Kubista E, Birner P, Oberhuber G. Overexpression of hypoxia-inducible factor 1alpha is associated with an unfavorable prognosis in lymph node-positive breast cancer. Clin Cancer Res 2002; 8: 1831-7.

[16] Birner P, Gatterbauer B, Oberhuber G, Schindl M, Rossler K, Prodinger A, Budka H, Hainfellner JA. Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer 2001; 92: 165-71.

[17] Yang QC, Zeng BF, Dong Y, Shi ZM, Jiang ZM, Huang J. Overexpression of hypoxia-inducible factor-1alpha in human osteosarcoma: correlation with clinicopathological parameters and survival outcome. Jpn J Clin Oncol 2007; 37: 127-34.

[18] Kontos CK, Papageorgiou SG, Diamantopoulos MA, Scorilas A, Bazani E, Vasilatou D, Gkontopoulos K, Glezou E, Stavroulaki G, Dimitriadis G, Pappa V. mRNA overexpression of the hypoxia inducible factor 1 alpha subunit gene (HIF1A): an independent predictor of poor overall survival in chronic lymphocytic leukemia. Leuk Res 2016; 53: 65-73.

[19] Hunter BA, Eustace A, Irlam JJ, Valentine HR, Denley H, Oguejiofor KK, Swindell R, Hoskin PJ, Choudhury A, West CM. Expression of hypoxia-inducible factor-1alpha predicts benefit from hypoxia modification in invasive bladder cancer. Br J Cancer 2014; 111: 437-43.

[20] Beasley NJ, Leek R, Alam M, Turley H, Cox GJ, Gatter K, Millard P, Fuggle S, Harris AL. Hypoxia-inducible factors HIF-1alpha and HIF-2alpha in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. Cancer Res 2002; 62: 2493-7.

[21] Evens AM, Sehn LH, Farinha P, Nelson BP, Raji A, Lu Y, Brakman A, Parimi V, Winter JN,

Schumacker PT, Gascoyne RD, Gordon LI. Hypoxia-inducible factor-1 {alpha} expression predicts superior survival in patients with diffuse large B-cell lymphoma treated with R-CHOP. J Clin Oncol 2010; 28: 1017-24.

[22] Powell JR, Dojcinov S, King L, Wosniak S, Gerry S, Casbard A, Bailey H, Gallop-Evans E, Maughan T. Prognostic significance of hypoxia inducible factor-1alpha and vascular endothelial growth factor expression in patients with diffuse large B-cell lymphoma treated with rituximab. Leuk Lymphoma 2013; 54: 959-66.

[23] Habermann TM. New developments in the management of diffuse large B-cell lymphoma. Hematology (Amsterdam, Netherlands) 2012; 17 Suppl 1: S93-7.

[24] Thieblemont C, Gisselbrecht C. Second-line treatment paradigms for diffuse large B-cell lymphomas. Curr Oncol Rep 2009; 11: 386-93.

[25] Zhou M, Wang J, Ouyang J, Xu JY, Chen B, Zhang QG, Zhou RF, Yang YG, Shao XY, Xu Y, Chen YM, Fan XS, Wu HY. MYC protein expression is associated with poor prognosis in diffuse large B cell lymphoma patients treated with RCHOP chemotherapy. Tumour Biol 2014; 35: 6757-62.

[26] Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, Scott DW, Tan KL, Steidl C, Sehn LH, Chan WC, Iqbal J, Meyer PN, Lenz G, Wright G, Rimsza LM, Valentino C, Brunhoeber P, Grogan TM, Braziel RM, Cook JR, Tubbs RR, Weisenburger DD, Campo E, Rosenwald A, Ott G, Delabie J, Holcroft C, Jaffe ES, Staudt LM, Gascoyne RD. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30: 3452-9.

[27] Horn H, Ziepert M, Becher C, Barth TF, Bernd HW, Feller AC, Klapper W, Hummel M, Stein H, Hansmann ML, Schmelter C, Moller P, Cogliatti S, Pfreundschuh M, Schmitz N, Trumper L, Siebert R, Loeffler M, Rosenwald A, Ott G. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 2013; 121: 2253-63.

[28] Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, Nielsen O, Gadeberg OV, Mourits-Andersen T, Frederiksen M, Pedersen LM, Moller MB. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30: 3460-7.

[29] Iqbal J, Meyer PN, Smith LM, Johnson NA, Vose JM, Greiner TC, Connors JM, Staudt LM, Rimsza L, Jaffe E, Rosenwald A, Ott G, Delabie J, Campo E, Braziel RM, Cook JR, Tubbs RR, Gascoyne RD, Armitage JO, Weisenburger DD, Chan WC. BCL2 predicts survival in germinal center B-cell-like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. Clin Cancer Res 2011; 17: 7785-95.

[30] Yang QP, Zhang WY, Yu JB, Zhao S, Xu H, Wang WY, Bi CF, Zuo Z, Wang XQ, Huang J, Dai L, Liu WP. Subtype distribution of lymphomas in Southwest China: analysis of 6,382 cases using WHO classification in a single institution. Diagn Pathol 2011; 6: 77.

[31] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Muller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-82.

[32] Xie Y, Bulbul MA, Ji L, Inouye CM, Groshen SG, Tulpule A, O'Malley DP, Wang E, Siddiqi IN. p53 expression is a strong marker of inferior survival in de novo diffuse large B-cell lymphoma and may have enhanced negative effect with MYC coexpression: a single institutional clinicopathologic study. Am J Clin Pathol 2014; 141: 593-604.

[33] Duyndam MC, van Berkel MP, Dorsman JC, Rockx DA, Pinedo HM, Boven E. Cisplatin and doxorubicin repress Vascular Endothelial Growth Factor expression and differentially down-regulate hypoxia-inducible factor I activity in human ovarian cancer cells. Biochem Pharmacol 2007; 74: 191-201.

[34] Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. EMBO J 2004; 23: 1949-56.

[35] Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci U S A 2000; 97: 9082-7.

[36] Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res 2001; 61: 6669-73.

[37] Tracy K, Dibling BC, Spike BT, Knabb JR, Schumacker P, Macleod KF. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. Mol Cell Biol 2007; 27: 6229-42.

[38] Podar K, Anderson KC. The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. Blood 2005; 105: 1383-95.

[39] Gratzinger D, Zhao S, Tibshirani RJ, Hsi ED, Hans CP, Pohlman B, Bast M, Avigdor A, Schiby G, Nagler A, Byrne GE Jr, Lossos IS, Natkunam

Y. Prognostic significance of VEGF, VEGF receptors, and microvessel density in diffuse large B cell lymphoma treated with anthracycline-based chemotherapy. Lab Invest 2008; 88: 38-47.

[40] Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ 2008; 15: 678-85.

[41] Giatromanolaki A, Koukourakis MI, Pezzella F, Sivridis E, Turley H, Harris AL, Gatter KC. Phosphorylated VEGFR2/KDR receptors are widely expressed in B-cell non-Hodgkin's lymphomas and correlate with hypoxia inducible factor activation. Hematol Oncol 2008; 26: 219-24.

[42] Wang Y, Ma J, Shen H, Wang C, Sun Y, Howell SB, Lin X. Reactive oxygen species promote ovarian cancer progression via the HIF-1alpha/LOX/E-cadherin pathway. Oncol Rep 2014; 32: 2150-8.

[43] Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, Kietzmann T, Gorlach A. Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. Arterioscler Thromb Vasc Biol 2007; 27: 755-61.

[44] Gupta D, Crosby ME, Almasan A, Macklis RM. Regulation of CD20 expression by radiation-induced changes in intracellular redox status. Free Radic Biol Med 2008; 44: 614-23.