Int J Clin Exp Pathol 2013;6(2):148-154
www.ijcep.com /ISSN:1936-2625/IJCEP1211002

Original Article

Amplified *RPS6KB1* and *CDC2* genes are potential biomarkers for aggressive HIV+/EBV+ diffuse large B-cell lymphomas

Xianfeng F Zhao[1], Merry Y Zhao[1], Ling Chai[2], Debra Kukuruga[1], Ming Tan[2], Sanford A Stass[1]

[1]*Department of Pathology,* [2]*Division of Biostatistics, Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA*

Received November 2, 2012; Accepted November 27, 2012; Epub January 15, 2013; Published February 1, 2013

Abstract: *RPS6KB1* encodes p70S6K/p85S6K, which plays a role in the PI3K/Akt/mTOR signal transduction pathway. *CDC2* gene encodes cdc2, which is critical for G2/M cell cycle progression. We had previously shown that amplified *RPS6KB1* and *CDC2* are commonly detected in the EBV+ diffuse large B-cell lymphoma (DLBCL) in HIV patients. In current study, we further evaluated the amplified *RPS6KB1* and *CDC2* genes in 12 HIV-related aggressive B-cell lymphomas and 10 non-HIV-related DLBCL using real time quantitative PCR. The cases were divided into 4 groups: 1) HIV-/EBV-; 2) HIV-/EBV+; 3) HIV+/EBV-; and 4) HIV+/EBV+. Receiver operating characteristic (ROC) curve and the area under the curve (AUC) was used to assess the ability of each gene to distinguish non-HIV+/EBV+ cases from HIV+/EBV+ cases. The AUC was estimated to be 0.76 for *RPS6KB1* and 0.74 for *CDC2* by using the Mann–Whitney statistic. Amplified *RPS6KB1* and *CDC2* genes were more frequently detected in common variants of DLBCL associated with HIV infection. Taken together, amplified *RPS6KB1* and *CDC2* are potential biomarkers for the aggressive DLBCL, particularly in HIV+/EBV+ patients. This study also suggests that the HIV+/EBV+ aggressive DLBCL could be potentially treated by targeting *RPS6KB1* and *CDC2* genes.

Keywords: RPS6KB1, CDC2, HIV, EBV, lymphoma, biomarker

Introduction

HIV-related lymphomas are defined as aggressive malignant lymphomas that develop in patients infected with HIV. It is estimated that 5-10% of all HIV-infected individuals will have lymphoma as either an initial or subsequent AIDS-defining condition [1]. The risk of patients with AIDS in developing lymphoma is almost 100 times greater than that of the general population [2, 3]. The risk of developing primary CNS lymphomas is even greater (>1,000 times) in patients infected with HIV [4]. Although the introduction of highly active antiretroviral therapy (HAART) markedly decreased the lymphoma incidence from 1066 to 390/100,000 person-years [5], the most recent data show that the incidence of non-Hodgkin lymphoma (NHL) is still much higher in patients infected with HIV than the general population [6].

Although lymphoma is considered a systemic disease and early detection seems to play a lesser role for the clinical management of systemic lymphomas than for the carcinomas, early intervention with intrathecal prophylaxis does decrease lymphoma involvement of the CNS [7]. Although diagnosis of AIDS precedes the onset of NHL in approximately 57% of the patients, in 30% of the patients the diagnosis of AIDS is made at the time of the diagnosis of NHL and HIV positivity [8]. Early detection of HIV-related lymphoma, which requires reliable biomarkers, will also prompt the high risk patients to be treated with early with HAART which prolong their lives.

RPS6KB1 encodes p70S6K, a downstream protein kinase in the PI3K/Akt/mTOR signal transduction pathway, where as *CDC2* encodes cdc2, a protein that plays key roles in the G2/M phase cell cycle progression. Regarded as oncogenes, amplified *RPS6KB1* and/or *CDC2* have been detected in a subset of diffuse large B-cell lymphomas (DLBCL), particularly in the HIV+/EBV+ DLBCL [9]. Since DLBCL comprises 75-80% of

the HIV-related NHL, this finding prompted us to hypothesize that amplified *RPS6KB1* and *CDC2* genes could be biomarkers for the early detection and clinical follow up of HIV-related EBV+ large B-cell lymphomas. We thus examined additional tissue samples of EBV+ DLBCL for amplified *RPS6KB1* and *CDC2* genes. Our data suggest that these amplified genes could be potential biomarkers for the aggressive DLBCLs, particularly in patients with HIV infections.

Materials and methods

Reagents

The QIAamp® DNA Mini Kit was purchased from Qiagen (Qiagen, Valencia, CA). The HPLC-purified oligonucleotide primers for real time quantitative PCR (qPCR) were synthesized by the University of Maryland School of Medicine Biopolymer Core Facility, and the iQTM SYBR® Green Supermix were from the BioRad Laboratories (Hercules, CA).

Patient specimens

With IRB approval, we have identified 12 in house cases of HIV-related large B-cell lymphoma from the 2005-2009 pathology archives of the University of Maryland Medical Center. Ten cases of non-HIV-related DLBCL were used as control. The in house cases were divided into 4 groups: 1) EBV+ DLBCL from HIV-infected patients (HIV+/EBV+); 2) EBV- DLBCL from HIV-infected patients (HIV+/EBV-); 3) EBV+ DLBCL from HIV-negative patients (HIV-/EBV+); and 4) EBV- DLBCL from HIV-negative patients (HIV-/EBV-). All the specimens were handled in the Biosafety Level II Labgard 425 Biological Safety Cabinet (NuAire, Plymouth, MN) with universal precaution.

Real-time quantitative PCR

Genomic DNA was isolated from formalin-fixed paraffin-embedded tissue sections using QIAamp® DNA Mini Kit (Qiagen). Serially diluted normal human genomic DNA was tested first by the real-time quantitative (q) PCR assay to generate a standard curve of the CT (cycle of threshold). Ten ng genomic DNA served as template for Singleplex real time qPCR analysis using a PCR master mix (containing 12.5 μL iQTM SYBR® Green Supermix (BioRad Laboratories, Hercules, CA), Two hundred nM forward primer and Two hundred nM reverse primer [9] to amplify a 135 bp DNA fragment of the first coding exon of *RPS6KB1* corresponding to nt 16623607-16623741 of human Chromosome 17 genomic contig (NT_010783.14). A 142 bp DNA fragment of the fifth coding exon of *CDC2* corresponding to nt 11103061-11103202 of human Chromosome 10 genomic contig (NT_008583.16) was similarly amplified. A 254 bp β-actin gene (*ACTB*) was concomitantly amplified for calibration. The PCR conditions were: 10 minutes at 95°C, then 10 seconds at 95°C and 60 seconds at 55°C for 40 cycles. The amplification levels were normalized to that of a normal control. Reactions were performed and analyzed on a 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). The relative gene amplification levels were calculated using the Livak 2-ΔΔCT method [10]. Based on our earlier experience, we used >5 fold amplification as the cutoff value for the low and high amplifications [9].

Statistics

Gene expression data were log transformed before analysis. Wilcoxon rank sum test was used for tests of difference between groups for independent samples. Wilcox signed rank test was used for tests for difference between variables for dependent samples. Fisher's exact test, Pearson's correlation or ANOVA was used to test relationships between two variables. The area under the Receiver operating characteristic (ROC) curve (AUC) was used to assess the ability of *RPS6KB1* and *CDC2*, respectively, to distinguish non-HIV+/EBV+ cases from HIV+/EBV+ cases. The AUC was estimated using the Mann–Whitney statistic.

Results

Amplified RPS6KB1 and CDC2 genes are frequently detected in the nodal EBV+ DLBCLs from patients with HIV infection

We have previously detected amplified *RPS6KB1* and *CDC2* genes in the nodal DLBCL, particularly in the EBV+ large B-cell lymphomas from HIV-infected patients [9]. To determine whether the amplified *RPS6KB1* and *CDC2* genes are indeed surrogates for the HIV-related EBV+ DLBCL, additional lymphoma specimens from our tumor bank were tested for the ampli-

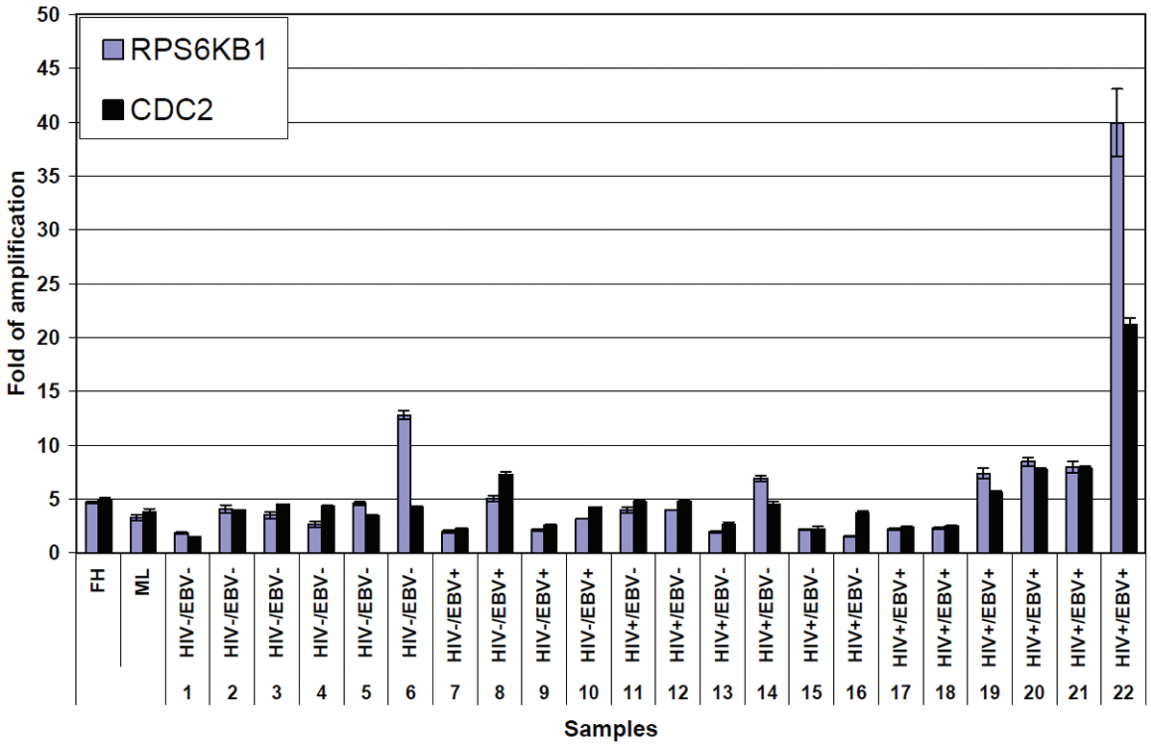

Figure 1. Detection of amplified *RPS6KB1* and *CDC2* genes in the HIV+/EBV+ DLBCL versus the non-HIV+/EBV+ DLBCL.

fied *RPS6KB1* and *CDC2* genes in 4 groups of DLBCL (**Table 1**). As shown in **Figure 1**, in the group of six DLBCL from patients without HIV or EBV infection, only a single case (case 6) showed amplified *RPS6KB1* (17%). One (case 8) of the four lymphomas (25%) in the group of patients with EBV but no HIV infection has amplified *RPS6KB1* and *CDC2*. In the six cases of patient group with HIV, but no EBV infection, only one (case 14) showed amplified *RPS6KB1* (17%), whereas four out of six EBV+ DLBCL (67%) from HIV+ patients showed both amplified *RPS6KB1* and *CDC2* genes. Statistical analysis indicated that the amplified *RPS6KB1* and *CDC2* can differentiate EBV+/HIV+ DLBCL from the other DLBCLs (see later section).

Amplified RPS6KB1 and CDC2 genes are associated with aggressive variants of DLBCL

Pathologically, the four EBV+ lymphomas with amplified *RPS6KB1* and *CDC2* genes from HIV+ patients are of anaplastic (cases 19 & 20) and plasmablastic (case 21 & 22) variants (**Figure 2B-D**), whereas the other 2 lymphomas (cases 6 & 8) with amplified *RPS6KB1* and *CDC2* genes are of immunoblastic variant by morphology (**Figure 2A**). Only one centroblastic DLBCL (case 14) has amplified *RPS6KB1* and *CDC2* genes. Furthermore, all the large B-cell lymphomas with amplified *RPS6KB1* and *CDC2* are negative for CD10, a marker indicating a good prognosis. Of note, one EBV+ peripheral T-cell lymphoma from an HIV-infected patient also showed amplified *RPS6KB1* and *CDC2* genes (not shown).

Amplified RPS6KB1 and CDC2 genes are significantly increased in the HIV+/EBV+ DLBCL

To evaluate whether the amplified *RPS6KB1* and *CDC2* genes can differentiate the HIV+/EBV+ DLBCL from the other DLBCLs, we compared the HIV+/EBV+ group with all the other groups lumped together as one non-HIV+/EBV+ group. Patients were younger in the HIV+/EBV+ group than in the non-HIV+/EBV+ group (p-value = 0.034). The amplified *RPS6KB1* and *CDC2* genes are highly positively correlated (Person's correlation r = 0.83, $p < 0.001$) (**Figure 3**).

The area under the Receiver operating characteristic (ROC) curve (AUC) was used to see how well the two biomarkers can distinguish the

Table 1. Clinical information of the patients with large B-cell lymphomas

Cases	Age/Sex	Biopsy site	Viral infection(s)	Lymphoma morphologic subtype	Immunophenotype	Treatment (with HAART)	Clinical status (survival time after diagnosis)
1	74/M	Iliac bone	HIV-/EBV-	DLBCL, NOS	CD20+, CD10+, BCL6+	R-EPOCH/R-CHOP[1]	Alive with CR (72 m)
2	58/F	LN	HIV-/EBV-	Lymphoplasmacytic	CD20+, CD10-, BCL6+	R-EPOCH	Died of disease (48 m)
3	72/M	Pancreas	HIV-/EBV-	Centroblastic DLBCL	CD20+, CD10-, BCL6+	R-CHOP	Lost for follow up
4	41/M	Mediastinum	HIV-/EBV-	Primary mediastinal	CD20+, CD10-, BCL6-	R-EPOCH	Alive with CR (72 m)
5	56/F	Adrenal	HIV-/EBV-	Centroblastic DLBCL	CD20+, CD10-, BCL6+	No document	Died of disease (48 m)
6	58/M	Groin	HIV-/EBV-	**Immunoblastic DLBCL**[2]	CD20+, CD10-, BCL6+	R-EPOCH/R-SHAP	Alive with CR (60 m)
7	43/M	Soft palatine	HIV-/EBV+	DLBCL, NOS	CD20+, CD10-, BCL6-	R-CHOP	Alive with CR (36 m)
8	70/M	Nasopharynx	HIV-/EBV+	**Immunoblastic DLBCL**	CD20+, CD10-, BCL6-	R-CHOP	Alive with CR (60 m)
9	70/M	Parotid	HIV-/EBV+	Centroblastic DLBCL	CD20+, ND	R-CHOP	Alive with CR (60 m)
10	61/M	Bone marrow	HIV-/EBV+	**Centroblastic DLBCL**	CD20+, CD10+, BCL6+	R-CHOP	Alive with CR (44 m)
11	58/M	Small intestine	HIV+/EBV-	DLBCL, Burkitt-like	CD20+, CD10+, BCL6+	Hyper-CVAD	Lost for follow up
12	31/M	Rectal	HIV+/EBV-	Centroblastic DLBCL	CD20+, CD10+, BCL6+	R-SHAP/Auto-SCT	Died of AIDS (24 m)
13	39/M	Epidural	HIV+/EBV-	DLBCL, NOS	CD20+, CD10+, BCL6-	No treatment	Died of PE (5 d)
14	46/M	Inguinal	HIV+/EBV-	**Centroblastic DLBCL**	CD20+, BCL6+, CD10-	No treatment	Died of disease (1 m)
15	55/M	Parotid	HIV+/EBV-	Centroblastic DLBCL	CD20+, BCL6+, CD10-	No document	Died of disease (1 m)
16	57/M	Neck	HIV+/EBV-	DLBCL, NOS	CD20+, BCL6+, CD10-	R-CHOP	Died of AIDS (7 m)
17	46/F	Axillary	HIV+/EBV+	DLBCL, Burkitt-like	CD20+, CD10+, BCL6+,	R-EPOCH	Died of disease (4 m)
18	42/M	Tonsil	HIV+/EBV+	Centroblastic DLBCL	CD20+, CD10+, BCL6+,	R-EPOCH	Died of AIDS (36 m)
19	50/M	Groin	HIV+/EBV+	**Anaplastic DLBCL**	CD20+, CD10-, BCL6+	R-EPOCH	Died of AIDS (21 m)
20	35/M	Neck	HIV+/EBV+	**Anaplastic DLBCL**	CD20+, CD138-	R-EPOCH	Died of disease (4 m)
21	33/M	Anal	HIV+/EBV+	**Plasmablastic lymphoma**	CD20-, CD10+, CD138+	CHOP	Died of disease (6 m)
22	51/F	Oral	HIV+/EBV+	**Plasmablastic lymphoma**	CD20-, CD10+, BCL6-	EPOCH/CHOP	Died of disease (6 m)

[1]CR, complete remission; CVAD, cyclophosphamide, vincristine, Adriamycin, and dexamethasone; ND, not done due to tissue exhaustion; R-CHOP, rituximab, cyclophosphamide, hydroxydaunomycin, vincristine sulfate (oncovin), and prednisone; R-EPOCH, rituximab, etoposide, prednisone, vincristine sulfate (oncovin), cyclophosphamide, and hydroxydaunomycin; R-SHAP, rituximab, methylprednisolone, cytarabine, cisplatin; SCT, stem cell transplant. [2]The lymphoma subtypes with amplified RPS6KB1 and/or CDC2 are in bold face.

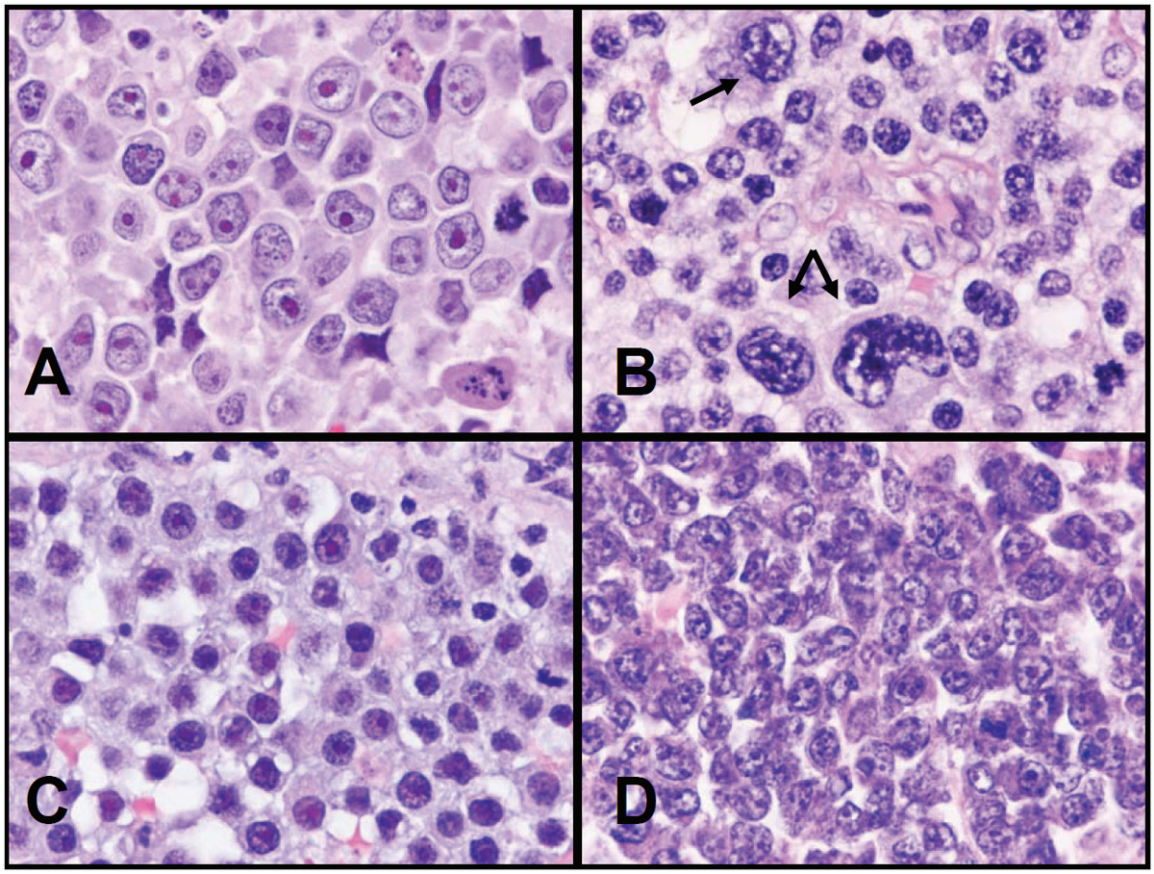

Figure 2. Morphology of the DLBCL variants with amplified *RPS6KB1* and *CDC2* genes. A: Immunoblastic variant (case 6); B: Anaplastic variant (case 20); C & D: Plasmablastic lymphoma (cases 21 & 22).

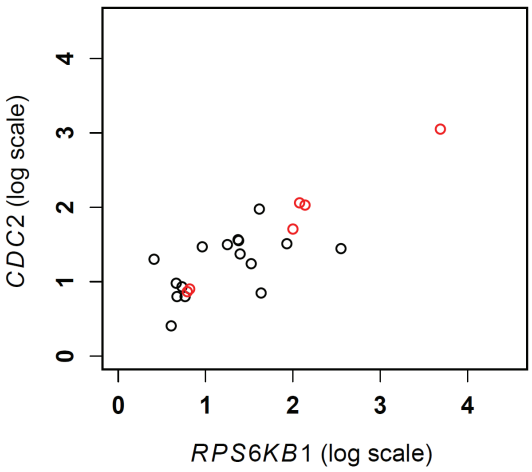

Figure 3. Correlation between *RPS6KB1* and *CDC2* in the DLBCLs (A: *Black* non-HIV+/EBV+; *Red*, HIV+/EBV+).

HIV+/EBV+ cases from the non-HIV+/EBV+ cases. Since *RPS6KB1* and *CDC2* are highly correlated, the performance for them is similar. The AUC was estimated to be 0.76 for *RPS6KB1* and 0.74 for *CDC2*.

Discussion

Our findings showed that the amplified *RPS6KB1* and *CDC2* genes can effectively (approximately 75% of the times) distinguish the HIV+/EBV+ from the non-HIV+/EBV+ aggressive DLBCLs. A strong correlation was also found between the amplified *RPS6KB1* and *CDC2* in each individual lymphoma specimen. Amplified *RPS6KB1* and *CDC2* genes are frequently identified in the aggressive variants of DLBCLs, including plasmablastic, immunoblastic, and anaplastic variants. Therefore, the current data support our hypothesis that the amplified *RPS6KB1* and *CDC2* genes are potential biomarkers for the HIV-related EBV+ DLBCLs.

HIV-related lymphomas can be divided into at least two distinct clinical categories based on

the sites of disease involvement. Approximately 80% of the HIV-related lymphomas arise in the periphery, and 20% occur in the central nervous system [11]. Histologically, 75-80% of these lymphomas are DLBCL, and the rest 20-25% are classified as Burkitt lymphomas (BL). The 2008 WHO Classification considers primary DLBCL of CNS and plasmablastic lymphoma as distinct clinical entities of DLBCL [12] based on their common association with compromised immune status, while the not otherwise specified (DLBCL-NOS) DLBCL are further classified morphologically into centroblastic, immunoblastic, anaplastic, and other rare variants. Recent studies suggest that the centroblastic variant has a better prognosis, whereas the immunoblastic variant has a worse prognosis [13]. The anaplastic variant is less well studied due to its rarity. However, since the large atypical neoplastic cells (see **Figure 2**) in the anaplastic variant are usually CD30+, they may represent immunoblasts or activated B cells; this variant thus may have a poor prognosis as well. Interestingly, the majority (approximately 86%) of the DLBCLs with amplified *RPS6KB1* and *CDC2* in our study are plasmablastic lymphomas, immunoblastic and anaplastic DLBCLs.

Except for the plasmablastic lymphoma that is a *bona fide* plasma cell neoplasm [14], all the B-cell lymphomas with amplified *RPS6KB1* and/or *CDC2* in this study are negative for CD10, a germinal center marker with good prognosis. This inverted trend for *RPS6KB1* and *CDC2* versus CD10 had been observed when we first identified the amplified *RPS6KB1* and *CDC2* genes in the index case of intravascular large B-cell lymphoma that relapsed from a nodal DLBCL, using array-based comparative genomic hybridization (CGH). In addition to the amplified *RPS6KB1* and *CDC2* genes, we also observed the deletion of CD10 and DAP-kinase genes in the recurrent intravascular large B-cell lymphoma [15].

The significance of amplified *RPS6KB1* and *CDC2* genes in the aggressive DLBCL is two folds. 1) Amplified *RPS6KB1* and *CDC2* genes can be the surrogates for detection of HIV-related large B-cell lymphomas in limited amount of specimens such as fine needle aspirates or small core biopsies that otherwise preclude a definitive diagnosis. Amplified *RPS6KB1* and *CDC2* genes in the lymphoid tissue from patients with HIV infection will increase the probability of malignant lymphoma and help to make clinical decisions as to prevention or treatment of lymphomas in those patients. 2) Since our previous study (9) showed that inhibition of p70S6K phosphorylation and cdc2 led to decreased growth of DLBCL cells, detection of the *RPS6KB1* and *CDC2* genes also provides an option in managing those patients with rapamycin and cdc2 inhibitors.

We recognize the limitations of our current study with data derived from a single institution with a small number of available HIV-related lymphoma specimens. The background of our real time quantitative PCR assay is still high, thus we have to use a 5 fold cutoff value. Although the current assay calibrates the levels of *RPS6KB1* and *CDC2* to the *ACTB* (β-actin gene), a housekeeping gene, it is not known whether *ACTB* gene could serve as a control in all the tissues. Our assay also showed that not all the HIV+/EBV+ DLBCLs had amplified *RPS6KB1* and *CDC2* genes, thus might have missed some cases (case 17 & 18). However, our assay could stratify the HIV+/EBV+ DLBCLs into the amplifiers and non-amplifiers; the non-amplifiers may not benefit from therapies targeted at p70S6K and/or cdc2. Despite the limitations, our preliminary findings suggest that the amplified *RPS6KB1* and *CDC2* genes are associated with aggressive large B-cell lymphomas, and are potential biomarkers that could distinguish HIV+/EBV+ lymphoma from the non-HIV+/EBV+ lymphoma and provide a molecular basis for targeted therapy. Nonetheless, large multi-institutional collaborative studies may be required to confirm the current findings.

Acknowledgement

This work is partially support by a NIH Grant NIH/NCI 3U24CA115091-04S1 (to SAS).

Conflict of interest statement

The authors declare no conflict of interest associated with this work.

Address correspondence to: Dr. X Frank Zhao, Department of Pathology, University of California San Diego, VAMC-San Diego, West 3051, 3350 La Jolla Village Drive, San Diego, CA 92161, USA. Tel: 858-5528585, Ext. 2465; Fax: 858-6423918; E-mail: Frank.Zhao2@va.gov

Reference

[1] Hamilton-Dutoit SJ, Pallesen G, Franzmann MB, Karkov J, Black F, Skinhøj P, Pedersen C. AIDS-related lymphoma. Histopathology, immunophenotype, and association with Epstein-Barr virus as demonstrated by in situ nucleic acid hybridization. Am J Pathol 1991; 138: 149-163.

[2] Gail MH, Pluda JM, Rabkin CS, Biggar RJ, Goedert JJ, Horm JW, Sondik EJ, Yarchoan R, Broder S. Projections of the incidence of non-Hodgkin's lymphoma related to acquired immunodeficiency syndrome. J Natl Cancer Inst 1991; 83: 695-701.

[3] Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet 1991; 337: 805-809.

[4] Flinn IW and Ambinder RF. AIDS primary central nervous system lymphoma. Curr Opin Oncol 1996; 8: 373.

[5] Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA. HIV/AIDS Cancer Match Study. AIDS-related cancer and severity of immunosuppression in persons with AIDS. J Natl Cancer Inst 2007; 99: 962-972.

[6] Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, Grigg R, Hylton T, Pawlish KS, McNeel TS, Goedert JJ. Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer 2008; 123: 187-194.

[7] Little RF, Pittaluga S, Grant N, Steinberg SM, Kavlick MF, Mitsuya H, Franchini G, Gutierrez M, Raffeld M, Jaffe ES, Shearer G, Yarchoan R, Wilson WH. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood 2003; 101: 4653-4659.

[8] Knowles DM, Chamulak GA, Subar M, Burke JS, Dugan M, Wernz J, Slywotzky C, Pelicci G, Dalla-Favera R, Raphael B. Lymphoid neoplasia associated with the acquired immunodeficiency syndrome (AIDS). The New York University Medical Center experience with 105 patients (1981-1986). Ann Intern Med 1988; 108: 744-753.

[9] Zhao MY, Auerbach A, D'Costa AM, Rapoport AP, Burger AM, Sausville EA, Stass SA, Jiang F, Sands AM, Aguilera N, Zhao XF. Phospho-p70S6K/p85S6K and cdc2/cdk1 are novel targets for diffuse large B-cell lymphoma combination therapy. Clin Cancer Res 2009; 15: 1708-1720.

[10] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.

[11] Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet 1991; 337: 805-809.

[12] Swerdlow SH, Campo E, Jaffe ES, Harris NL, Pileri SA, Stein H, Thiele J, Vardiman JW (Eds.): World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon: IARC. 2008.

[13] Stein H and Hummel M. Histopathology in the light of molecular profiling. Ann Oncol 2006; 17 Suppl 4: iv5-7.

[14] Tavora F, Gonzalez-Cuyar LF, Sun CC, Burke A, Zhao XF. Extra-oral plasmablastic lymphoma: report of a case and review of literature. Hum Pathol 2006; 37: 1233-1236.

[15] Zhao XF, Nowak NJ, Conroy JM, Bartos JD, Anderson GR, Sait SN, Sands AM, and Barcos MP. Genetic evidence of evolution from diffuse to intravascular large B-cell lymphoma. The 43rd Annual Meeting of American Society of Hematology (Orlando, FL, December 2001). Abstract #4675.