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Original Article

Intravascular large B-cell lymphoma: report of three cases and analysis of the mTOR pathway

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Abstract: Intravascular large B-cell lymphoma (IVLBCL) is a rare, aggressive and often fatal non-Hodgkin lymphoma characterized by preferential growth of malignant B-cells within the lumina of small vessels. Rituximab plus anthracycline-based chemotherapy is the current standard regimen for IVLBCL, however it has minimal efficacy in relapsed or refractory diseases. Recent clinical trials have shown a significant anti-lymphoma activity of mammalian target of rapamycin (mTOR) inhibitors in relapsed and refractory diffuse large B-cell lymphoma (DLBCL); however, the activation status of the mTOR pathway and the therapeutic potential of mTOR inhibitors in IVLBCL have not yet been studied. Here we described the clinicopathological features of 3 cases of IVLBCL diagnosed at our institutions, and evaluated the activation status of the mTOR signaling in these tumors. Our results showed that the mTOR complex 2 pathway was selectively upregulated in IVLBCL, as evidenced by a predominant nuclear localization of the activated form of mTOR (p-mTOR at Ser2448) with concomitant overexpression of nuclear p-Akt (Ser473) and vascular endothelial growth factor (VEGF)-A in the lymphoma cells. These data suggest that overactivation of mTOR pathway may play a role in lymphomagenesis of IVLBCL and mTORC2 inhibitors may be beneficial in treating IVLBCL.

Keywords: Intravascular large B-cell lymphoma, mTOR, Akt, VEGF

Introduction

Intravascular large B-cell lymphoma (IVLBCL) is a rare and aggressive non-Hodgkin lymphoma characterized by almost exclusive malignant B-cell infiltrate within the lumina of small blood vessels, particularly capillaries [1]. It is now recognized as a separate disease entity, distinct from the diffuse large B-cell lymphoma (DLBCL), in the current WHO classification [2]. The mechanism for this vessel tropism is largely unknown. Absence of adhesion molecules including b1 integrin and ICAM-1 and metalloproteinases on the lymphoma cells may cause defective transvascular penetration and impaired degradation of extracellular matrix to form tumor masses [3], [4]. Aggregation of neoplastic B -cells occludes the lumina of small vessels, which may result in multiorgan ischemic injury clinically.

Owing to its unique intravascular and disseminated growth pattern, IVLBCL may virtually affect any sites and manifest as nonspecific clinical syndromes. Clinically, there are two variants: Western variant commonly presents as skin rashes and multiple neurological deficits, while patients from Asian countries preferentially show hemophagocytic syndrome, bone marrow involvement, and fever of unknown origin (FUO) [1]. Lymph node and peripheral blood involvements are rare, occurring in only 4-17% and 0-13% of cases, respectively [5]. Elevations in lactate dehydrogenase (LDH) and b2-microglobulin, and anemia are present in the majority of patients [6]. Due to its nonspecific clinical manifestations, IVLBCL is often a diagnostic challenge. As a matter of fact, nearly half of the cases are discovered in postmortem examination [7]. A definitive diagnosis requires histopathological demonstration of malignant B lymphocytes within the vessel lumina. Recently, several studies have proposed random skin biopsies in patients with FUO and nonspecific

syndromes including mental status changes and central nervous system (CNS) syndrome with or without skin lesions for a prompt diagnosis of IVLBCL [8].

Adding rituximab to the anthracycline-based chemotherapy such as CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone) (R-CHOP) has significantly improved the previously dismal prognosis of IVLBCL [9], [10]. However, a substantial proportion of patients eventually experience relapse, particularly in the brain, and R-CHOP has minimal efficacy in these patients [11].

Recently, clinical studies have demonstrated a significant anti-tumor activity of mammalian target of rapamycin (mTOR) inhibitors in relapsed DLBCL [12], [13]. However, the activation status of the mTOR pathway and the potential therapeutic role of mTOR inhibitors in IVLBCL have not yet been assessed. In this study, we describe the clinicopathological features of 3 cases of IVLBCL diagnosed at our institutions, and investigate the activation status of the mTOR signaling in these tumors.

Materials and methods

Study groups

The pathology database at Lyndon B. Johnson Hospital and Memorial Hermann Hospital at Texas Medical Center from 2003 to 2011 was retrospectively reviewed and a total of 3 cases of IVLBCL were identified. 16 tissue samples of reactive lymph nodes were also retrieved from the database. The tissue microarray block was subsequently created by manually re-embedding archived paraffin-embedded tissues from the reactive lymph nodes as previously described [14]. This study was conducted according to the institutional review board-approved research protocol.

Immunohistochemical analysis and scoring

The avidin-biotin peroxidase technique was used, using an autostainer [14] with primary antibodies against CD3, CD5, CD20, cyclin D1, Bcl-6, MUM1, Ki-67 (DAKO, Carpinteria, CA), vascular endothelial growth factor (VEGF)-A, phosphorylated (p)-mTOR at serine 2448, and p-Akt at serine 473 (Cell Signaling Technology, Inc., Beverly, MA). Primary antibodies against p-mTOR and p-Akt were incubated at 4°C, and all other primary antibodies were incubated at room temperature. Positive and negative controls were run in parallel with the samples. The expression of p-mTOR, p-Akt, and VEGF-A protein analytes were assessed using bright-field microscopy with regard to the following parameters: 1) Cellular compartmentalization of the chromogenic signal was indicated as predominantly cytoplasmic or nuclear. 2) Percentage of positive cells from 1 to 100% was determined in each individual case. 3) Qualitative assessment of chromogenic signal intensity was graded as absent (0), mild (1+), moderate (2+), or strong (3+). An expression index (EI) was calculated by taking the product of intensity score and percentage of positively staining cells.

Flow cytometric analysis

Immunophenotypic analysis of peripheral blood (Case 3) and bone marrow aspirate (Case 1 and Case 2) was performed using the whole blood lysis method, followed by 4-color flow cytometric analysis on a FACSort analyzer (Becton Dickinson, San Jose, CA) using the CD45 versus side-scatter gating strategy. Monoclonal antibodies, conjugated to fluorescein isothiocyanate, phycoerythrin, allophycocyanin, or peridinin chlorophyll protein were used, specific for the following antigens: CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD14, CD16, CD19, CD20, CD22, CD23, CD25, CD33, CD34, CD45, CD56, human leukocyte antigen (HLA)-DR, terminal deoxynucleotidyl transferase (TdT), and surface immunoglobulin kappa and lambda light chains. All antibodies were supplied by Becton Dickinson, Franklin Lakes, NJ.

Statistical analysis

Student t-test was used to determine significance of differences in expression index between IVLBCL and reactive lymph nodes. $P < 0.05$ was considered as statistically significant.

Results

Clinical findings

Case 1: A 56-year-old male with a history of transient ischemic attack and protein C and S deficiency was transferred from an outside hos-

pital with a presumed diagnosis of stroke. He complained of progressive left arm weakness and tingling, memory and bilateral hearing loss, and confusion for six months, which was markedly exacerbated one week prior to admission. Neurological examination was remarkable for fluctuating mental status, left eye deviation, nystagmus of the right eye, and bilateral Babinski's sign. Laboratory evaluation showed thrombocytopenia (85 x 10^6/dL), anemia (hemoglobin 10.0 g/dL), hypoalbuminemia (2.9 g/dL), and a slightly elevated level of serum LDH (224 U/L). MRI of the brain demonstrated multiple small infarcts, and predominantly periventricular and subcortical white matter lesions with subtle ill-defined areas of enhancement. These abnormal radiological findings may be caused by ischemic vasculopathy/vasculitis, neurotoxic changes, or lymphoma. Electroencephalography showed generalized slowing without epileptiform discharges. CT of the abdomen showed a 22-cm spleen but no lymphadenopathy. All other work-up including echocardiogram, cerebrospinal fluid analysis, and bone marrow biopsy were essentially unremarkable. A brain biopsy was performed. Histopathologic examination revealed intravascular large B-cell lymphoma cells within the brain capillaries, confirmed by immunohistochemical stains. The patient received six courses of R-CHOP. Despite improvement of neurological symptoms, he presented new onset of lymphadenopathies by CT. Eventually, he underwent autologous bone marrow transplant (BMT) at an outside hospital. Unfortunately, the lymphoma relapsed 2 years post-BMT. He then received a second BMT from a matched unrelated donor, and died of graft-versus-host disease 3 years after initial presentation.

Case 2: A 69-year-old male presented with a 1-month history of weakness, slurred speech, episodes of loss of consciousness, and a 15-pound weight loss. On admission, physical examination revealed no additional findings. Relevant laboratory data included increased levels of serum LDH (400 U/L) and b2-microglobulin (2.7 g/mL), and an unremarkable CBC. MRI of the brain showed nonspecific parenchymal abnormalities in the supratentorial regions and the corpus callosum, raising a broad differential diagnosis including vasculitis, drug-related changes, neurotoxic exposure, encephalitis, or neurodegenerative diseases. Brain biopsies were performed and revealed features of IVLBCL involving both cortex and white matter. Multiple enlarged retroperitoneal, retrocrural lymph nodes and splenic masses were appreciated on staging CT. Bone marrow aspirate and biopsy showed no evidence of lymphoma infiltrates. He was given high-dose intravenous methotrexate and discharged to an outside hospital for further therapy.

Case 3: This case has been previously reported [15]. A 51-year-old female came to the emergency room with fever, abdominal pain, nausea and vomiting for 5 days. She also complained of one-year history of generalized fatigue and a 50-pound weight loss. Except for right upper quadrant abdominal pain, the physical examination findings were unremarkable; no appreciable lymphadenopathy was noted. An abdominal CT showed gallbladder wall thickening and fluid. Significant laboratory data included pancytopenia (white blood cell count 2.2 x 10^6/dL, hemoglobin 10.7 g/dL, and platelet count 43 x 10^6/dL), increased serum LDH level (4,539 U/L), and low serum albumin level (2.1 g/dL). An initial diagnosis of acute cholecystitis was made. She underwent cholecystectomy. Histopathologic examination of the gallbladder revealed extensive involvement by IVLBCL. Flow cytometric analysis of the peripheral blood showed a small population of monoclonal large B-cells with immunophenotypes identical to those seen in the gallbladder. R-CHOP was given but was interrupted after one course due to profound neutropenic sepsis. The patient died of multiorgan failure 21 days after the diagnosis.

Pathological findings

Examination of the tissues from all 3 patients showed similar features: aggregates of atypical large lymphocytes completely occluded the lumina of small sized blood vessels (**Figure 1A, 1B**). The lymphoma cells were large, with vesicular chromatin, irregular nuclear contour, and single or multiple small nucleoli. Mitotic figures were easily seen, with Ki-67 proliferation rates of more than 90% in all three cases (**Figure 1C**). Immunohistochemical analysis revealed intense homogenous CD20 staining (**Figure 1D**) and lack of immunoreactivity for CD3. The neoplastic cells were also positive for Bcl-6 (weak nuclear stain; image not shown) and MUM1 (**Figure 1E**) in all cases. CD10 was negative in case 3 (no tissues were available in case 1 and case 2 for CD10 immunostain). These findings

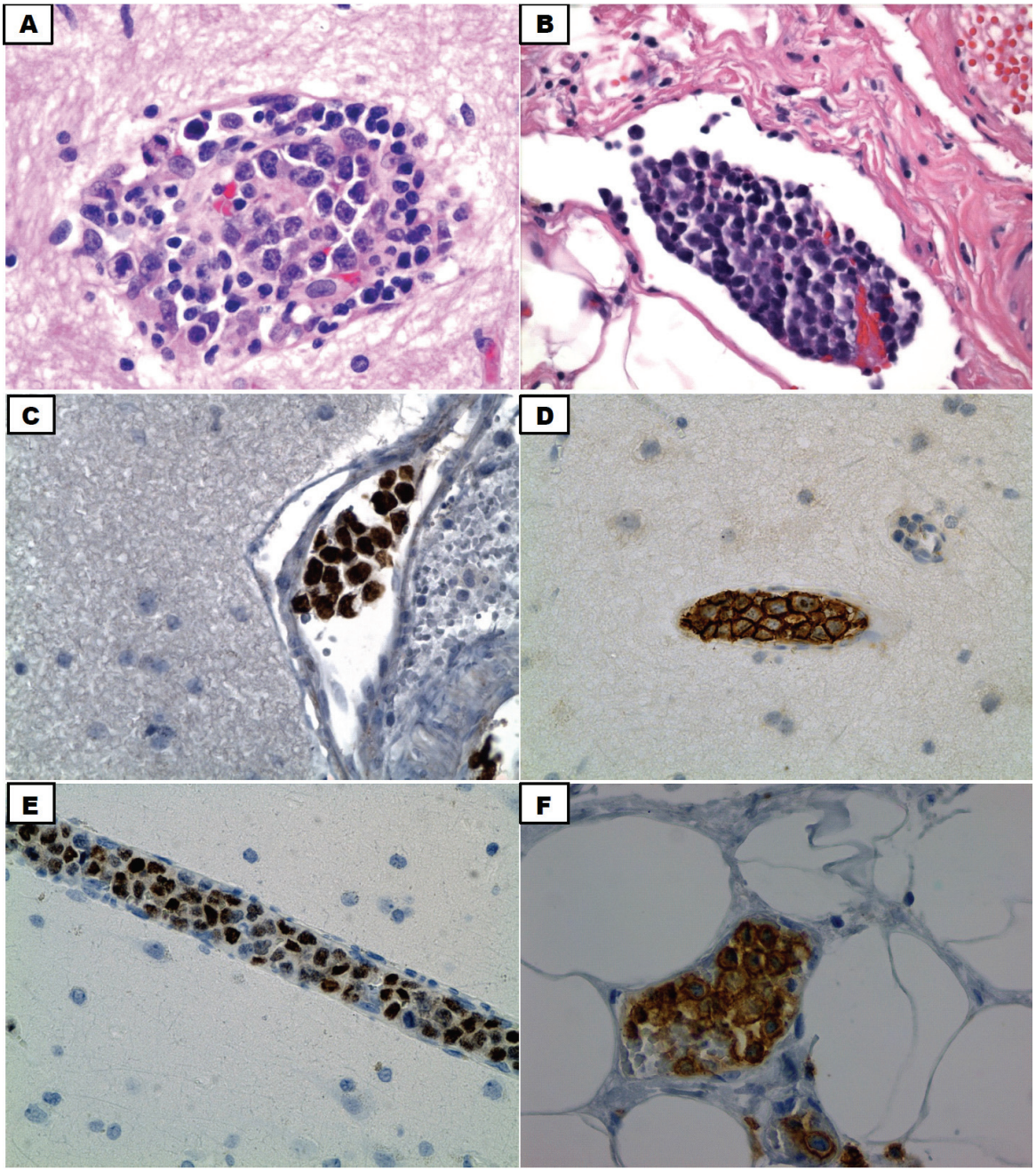

Figure 1. Histopathologic findings of IVLBCL. Brain biopsy from Case 1 (A) and gallbladder from Case 3 (B) showed obliteration of small blood vessels by aggregates of large atypical lymphoid cells. (C) Proliferation index was high by Ki -67 staining (Case 1). The neoplastic cells demonstrated intense staining of CD20 (Case 1, D) and strong nuclear staining of MUM1 (Case 2, E). In a subset of IVLBCL, the tumor cells showed coexpression of CD5 (Case 3, F); however immunostaining for CD23 and cyclin D1 was consistently negative (not shown). Original magnification x 400.

were consistent with a non-germinal center B-cell-like (non-GCB) phenotype in all 3 cases.

In case 3, flow cytometric analysis was also performed on peripheral blood, which identified a monoclonal B-cell population with immunophenotype similar to those in the gallbladder tissue. The lymphoma B-cells in case 3 also coexpressed CD5 (**Figure 1F**); however, lack of immunostaining for cyclin D1 and TdT argued

against a diagnosis of mantle cell lymphoma blastoid variant and lymphoblastic lymphoma, respectively [1].

Investigation of mTOR-Akt signal pathway

In the reactive lymph nodes, strong nuclear expression of p-mTOR was detected in a subset of germinal center B lymphocytes. Within the perifollicular area, nearly half of the reactive lymph nodes were immunonegative for p-mTOR, and less than 1/3 of cases had weak to moderate nuclear p-mTOR expression. In contrast, nuclear staining of p-mTOR was detected in all 3 IVLBCL cases, with 2 cases showing strong expression of p-mTOR (**Figure 2A, 2B**). The expression index (EI) of nuclear p-mTOR was significantly higher in IVLBCL compared to the perifollicular lymphocytes in non-neoplastic controls (300 versus 38 for IVLBCL and reactive lymph nodes, respectively, p = 0.001, **Table 1**). These data suggested a selective overactivation of mTOR complex 2 (mTORC2) in IVLBCL.

mTORC2 activates the downstream survival kinase Akt by phosphorylation at serine 473 [16]. Expression of p-Akt was also confined to the nucleus of lymphocytes. 2 of 3 IVLBCL showed strong nuclear p-Akt immunopositivity, whereas only 3 of 16 reactive lymph nodes revealed moderate to strong nuclear p-Akt staining in the perifollicular lymphocytes (**Figure 2C, 2D**). The level of nuclear p-Akt EI was 2-fold higher in the lymphoma cells than in non-neoplastic controls (187 versus 87 for IVLBCL and reactive lymph nodes, respectively, p = 0.29, **Table 1**); however, this difference failed to achieve statistical significance, probably due to the small sample size (n = 3) in IVLBCL.

VEGF-A is a target gene of the mTORC2 signaling via hypoxia-inducible factor-2alpha (HIF-2a) [17]. The expression of VEGF-A was seen in the plasmalemmal and cytoplasmic fractions in both groups. Intravascular lymphomas showed significantly increased EI of VEGF-A compared to the perifollicular lymphocytes in reactive lymph nodes (174 versus 75 for IVLBCL and reactive lymph nodes, respectively, p = 0.02, **Figure 2E, 2F**, **Table 1**).

Discussion

IVLBCL is a rare and often fatal systemic neoplasm. Its poor prognosis might be attributed by multiple factors. First, it is difficult to diagnose due to its rarity, highly variable and nonspecific clinical presentations, and often lack of nodal or extranodal masses. As a consequence, initiation of chemotherapy is often delayed. Second, the majority of IVLBCL patients are elderly (median age, 67 years) with poor performance status, which make it difficult to implement adequate chemotherapy. Furthermore, 80% of IVLBCL cases have biologically unfavorable phenotypes as activated B-cell (ABC) type [18], and about 30% of IVLBCL cases are CD5-positive [19], which is known to be associated with significantly decreased survival rate in patients with DLBCL. These findings are identical to our studies: all 3 cases are ABC type, and the tumor cells in 1 of 3 cases expressed CD5 [20].

CHOP or CHOP-like anthracycline-containing chemotherapy was the mainstay of treatment for IVLBCL in the pre-rituximab era, however its clinical efficacy was disappointing [21]. Rituximab remarkably altered the dismal natural history of IVLBCL. With the addition of rituximab, the 2-year overall survival was increased from 46% to 66% in European patients [9], and an even more pronounced improvement was also observed in Asian patients [22]. However, despite the high initial remission rates, a signifi-

Table 1. Average expression index (EI) of mTOR pathway markers in IVLBCL and reactive lymph nodes

	Nuclear p-mTOR	Nuclear p-AKT	VEGF-A
IVLBCL	300*	187	174*
Post-Germinal Center Lymphocytes in Reactive Lymph Nodes	38	87	75

*Significant difference in IVLBCL compared to reactive lymph nodes; 3 cases in IVLBCL and 16 cases in reactive lymph nodes.

Abbreviations: IVLBCL, intravascular large B-cell lymphoma; mTOR, mammalian target of rapamycin; VEGF-A, vascular endothelial growth factor-A.

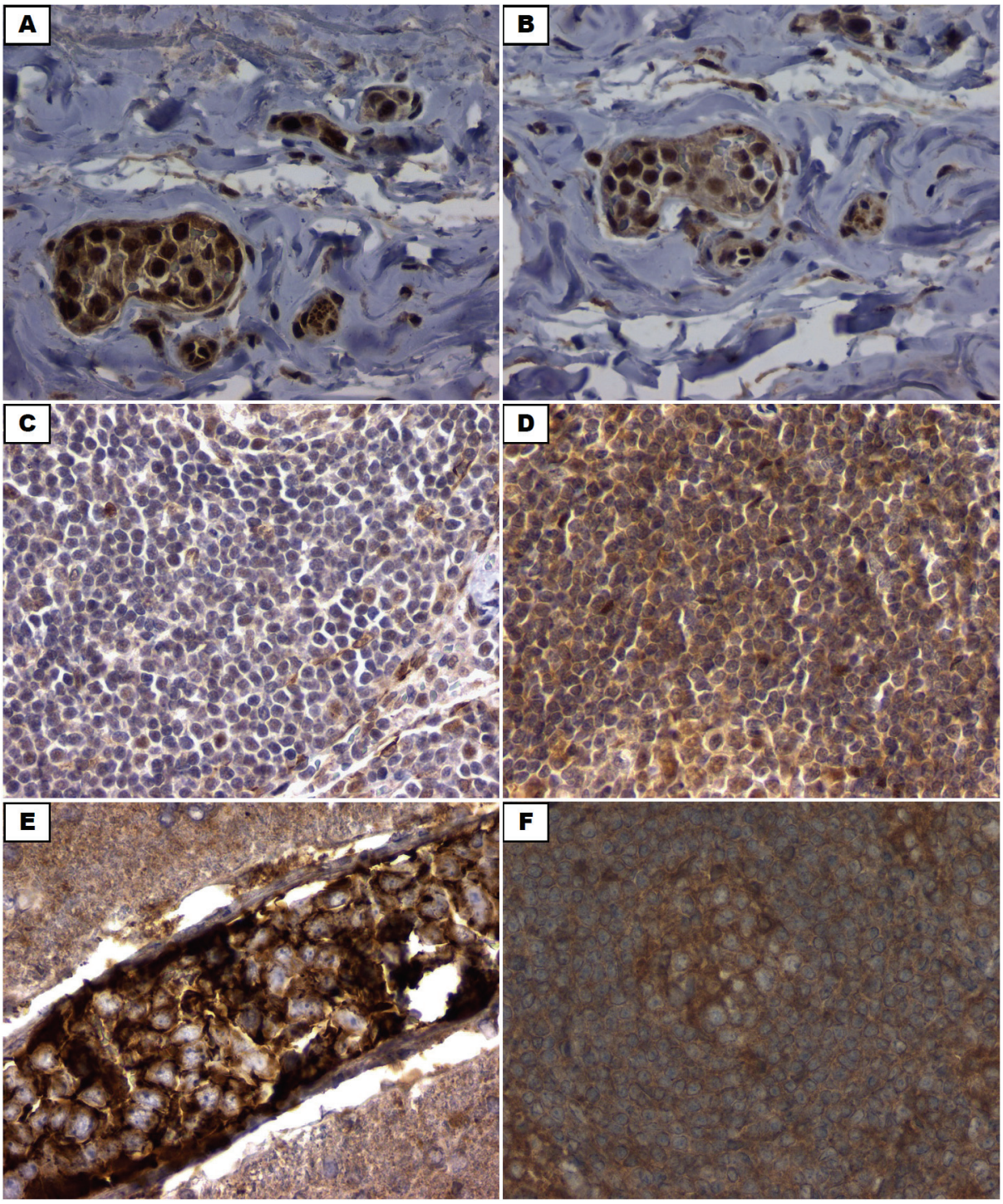

Figure 2. Expression of the mTOR pathway markers in IVLBCL and reactive lymph nodes. A-D: Phosphospecific antibodies against p-mTOR at serine 2448 and p-Akt at serine 473 showed chromogenic signals predominantly confined to the nucleus. The expression index (EI) of nuclear p-mTOR and p-Akt was significantly higher in IVLBCL compared to the perifollicular lymphocytes in non-neoplastic controls. (A) p-mTOR (Ser2448) in IVLBCL (Case 3); (B) p-mTOR (Ser2448) in reactive lymph nodes; (C) p-Akt (Ser473) in IVLBCL (Case 3); (D) p-Akt (Ser473) in reactive lymph nodes. E and F: Antibodies recognizing VEGF-A showed signals in the plasmalemmal/cytoplasmic fractions of lymphocytes. IVLBCL had significantly increased EI of VEGF-A compared to the perifollicular lymphocytes in non-neoplastic lymph nodes (E) VEGF-A in IVLBCL (Case 2); (F) VEGF-A in reactive lymph nodes. Original magnification x 400.

cant percentage of patients, 62.5% in one recent report [11], eventually relapsed, particularly in the patients with CNS involvement. Once relapsed, response was hardly achieved with R-CHOP or other currently available salvage therapies. In our series, of the two patients who received R-CHOP, both experienced treatment failure: one with high tumor burden died of disease shortly after diagnosis, while the other developed disease progression following 6 courses of R-CHOP. Therefore, new therapeutic strategies are clearly needed for IVLBCL, especially those with refractory or relapsed diseases. One promising class of such anticancer drugs might be the mTOR inhibitors.

mTOR signaling has an essential role in the regulation of cell growth and survival [23]. It assembles into two complexes (mTORC): mTORC1 is predominantly cytoplasmic and sensitive to rapamycin; in contrast, mTORC2 is both cytoplasmic and nuclear, and relatively resistant to rapamycin [23], [24]. Upon activation, mTORC1 and mTORC2 phosphorylatively regulate their respective downstream effectors p70S6K/4EBP1, and Akt [16]. High levels of dysregulated mTOR activity are associated with many human diseases, including lymphomagenesis [25], whereas mTOR inhibitors have exhibited efficacies against advanced lymphoma cells both in vitro and in vivo. For example, treatment with everolimus induces cell cycle arrest and augments the cytotoxic effects of rituximab and anthracycline in DLBCL cell lines [26]. In addition, single-agent everolimus or temsirolimus has shown an overall response rate of 28% to 35% in three phase II trials for heavily treated refractory/relapsed DLBCL patients [27]. These positive findings in DLBCL prompted us to perform a morphoproteomic analysis of the mTOR signaling in IVLBCL. Our study has revealed the overexpression of the following protein analytes in IVLBCL versus the perifollicular B lymphocytes in their non-neoplastic counterpart: nuclear p-mTOR (Ser2448), nuclear p-Akt (Ser473), and VEGF-A. This data suggests that the rapamycin-insensitive mTORC2 signaling might be involved in the tumorigenesis of IVLBCL, and therapies targeting this pathway might be beneficial in treating IVLBCL.

A rapalogue such as rapamycin or everolimus, or temsirolimus would most likely not be very effective in IVLBCL if used by itself due to potentially enhanced activation of the mTORC2 target Akt, causing rapamycin resistance [28]. If a rapalogue is considered for treatment in this type of lymphoma, addition of a histone deacetylase inhibitor (HDAI) [29], should overcome rapamycin resistance by blocking mTORC2, thus preventing Akt activation [28]. HDAI agents have shown promising results in recurrent and refractory lymphomas [30].

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References

[1] Ponzoni M, Ferreri AJ, Campo E, Facchetti F, Mazzucchelli L, Yoshino T, Murase T, Pileri SA, Doglioni C, Zucca E, Cavalli F and Nakamura S. Definition, diagnosis, and management of intravascular large B-cell lymphoma: proposals and perspectives from an international consensus meeting. J Clin Oncol 2007; 25: 3168-3173.

[2] Swerdlow S (Editor), Campo E (Editor), Harris NL (Editor), Jaffe ES (Editor), Pileri SA (Editor), Stein H (Editor), Thiele J (Editor), Vardiman JW (Editor). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue, 4th ed (World Health Organization; Fourth Edition). Lyon, 2008; 252-253

[3] Kinoshita M, Izumoto S, Hashimoto N, Kishima H, Kagawa N, Hashiba T, Chiba Y and Yoshimine T. Immunohistochemical analysis of adhesion molecules and matrix metalloproteinases in malignant CNS lymphomas: a study comparing primary CNS malignant and CNS intravascular lymphomas. Brain Tumor Pathol 2008; 25: 73-78.

[4] Ponzoni M, Arrigoni G, Gould VE, Del Curto B, Maggioni M, Scapinello A, Paolino S, Cassisa A and Patriarca C. Lack of CD 29 (beta1 integrin) and CD 54 (ICAM-1) adhesion molecules in intravascular lymphomatosis. Hum Pathol 2000; 31: 220-226.

[5] Ferreri AJ, Dognini GP, Campo E, Willemze R,

Seymour JF, Bairey O, Martelli M, De Renz AO, Doglioni C, Montalban C, Tedeschi A, Pavlovsky A, Morgan S, Uziel L, Ferracci M, Ascani S, Gianelli U, Patriarca C, Facchetti F, Dalla Libera A, Pertoldi B, Horvath B, Szomor A, Zucca E, Cavalli F and Ponzoni M. Variations in clinical presentation, frequency of hemophagocytosis and clinical behavior of intravascular lymphoma diagnosed in different geographical regions. Haematologica 2007; 92: 486-492.

[6] Ponzoni M and Ferreri AJ. Intravascular lymphoma: a neoplasm of 'homeless' lymphocytes? Hematol Oncol 2006; 24: 105-112.

[7] Domizio P, Hall PA, Cotter F, Amiel S, Tucker J, Besser GM and Levison DA. Angiotropic large cell lymphoma (ALCL): morphological, immunohistochemical and genotypic studies with analysis of previous reports. Hematol Oncol 1989; 7: 195-206.

[8] Asada N, Odawara J, Kimura S, Aoki T, Yamakura M, Takeuchi M, Seki R, Tanaka A and Matsue K. Use of random skin biopsy for diagnosis of intravascular large B-cell lymphoma. Mayo Clin Proc 2007; 82: 1525-1527.

[9] Ferreri AJ, Dognini GP, Bairey O, Szomor A, Montalban C, Horvath B, Demeter J, Uziel L, Soffietti R, Seymour JF, Ambrosetti A, Willemze R, Martelli M, Rossi G, Candoni A, De Renzo A, Doglioni C, Zucca E, Cavalli F and Ponzoni M. The addition of rituximab to anthracycline-based chemotherapy significantly improves outcome in 'Western' patients with intravascular large B-cell lymphoma. Br J Haematol 2008; 143: 253-257.

[10] Ferreri AJ, Dognini GP, Govi S, Crocchiolo R, Bouzani M, Bollinger CR, D'Incan M, Delaporte E, Hamadani M, Jardin F, Martusewicz-Boros M, Montanari M, Szomor A, Zucca E, Cavalli F and Ponzoni M. Can rituximab change the usually dismal prognosis of patients with intravascular large B-cell lymphoma? J Clin Oncol 2008; 26: 5134-5136; author reply 5136-5137.

[11] Masaki Y, Dong L, Nakajima A, Iwao H, Miki M, Kurose N, Kinoshita E, Nojima T, Sawaki T, Kawanami T, Tanaka M, Shimoyama K, Kim C, Fukutoku M, Kawabata H, Fukushima T, Hirose Y, Takiguchi T, Konda S, Sugai S and Umehara H. Intravascular large B cell lymphoma: proposed of the strategy for early diagnosis and treatment of patients with rapid deteriorating condition. Int J Hematol 2009; 89: 600-610.

[12] Smith SM, van Besien K, Karrison T, Dancey J, McLaughlin P, Younes A, Smith S, Stiff P, Lester E, Modi S, Doyle LA, Vokes EE and Pro B. Temsirolimus has activity in non-mantle cell non-Hodgkin's lymphoma subtypes: The University of Chicago phase II consortium. J Clin Oncol 2010; 28: 4740-4746.

[13] Witzig TE, Reeder CB, LaPlant BR, Gupta M, Johnston PB, Micallef IN, Porrata LF, Ansell SM, Colgan JP, Jacobsen ED, Ghobrial IM and Habermann TM. A phase II trial of the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. Leukemia 2011; 25: 341-347.

[14] Shen Q, Stanton ML, Feng W, Rodriguez ME, Ramondetta L, Chen L, Brown RE and Duan X. Morphoproteomic analysis reveals an overexpressed and constitutively activated phospholipase D1-mTORC2 pathway in endometrial carcinoma. Int J Clin Exp Pathol 2010; 4: 13-21.

[15] Duan X, Lapus A, Brown RE and Chen L. Intravascular large B-cell lymphoma presenting as cholecystitis and pancytopenia: case report with literature review. Ann Clin Lab Sci 2011; 41: 301-305.

[16] Holz MK, Ballif BA, Gygi SP and Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell 2005; 123: 569-580.

[17] Brugarolas JB, Vazquez F, Reddy A, Sellers WR and Kaelin WG, Jr. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 2003; 4: 147-158.

[18] Kanda M, Suzumiya J, Ohshima K, Tamura K and Kikuchi M. Intravascular large cell lymphoma: clinicopathological, immunohistochemical and molecular genetic studies. Leuk Lymphoma 1999; 34: 569-580.

[19] Murase T, Yamaguchi M, Suzuki R, Okamoto M, Sato Y, Tamaru J, Kojima M, Miura I, Mori N, Yoshino T and Nakamura S. Intravascular large B-cell lymphoma (IVLBCL): a clinicopathologic study of 96 cases with special reference to the immunophenotypic heterogeneity of CD5. Blood 2007; 109: 478-485.

[20] Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, Ott G, Rosenwald A, Braziel RM, Campo E, Vose JM, Lenz G, Staudt LM, Chan WC and Weisenburger DD. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol 2011; 29: 200-207.

[21] Ferreri AJ, Campo E, Ambrosetti A, Ilariucci F, Seymour JF, Willemze R, Arrigoni G, Rossi G, Lopez-Guillermo A, Berti E, Eriksson M, Federico M, Cortelazzo S, Govi S, Frungillo N, Dell'Oro S, Lestani M, Asioli S, Pedrinis E, Ungari M, Motta T, Rossi R, Artusi T, Iuzzolino P, Zucca E, Cavalli F and Ponzoni M. Anthracycline-based chemotherapy as primary treatment for intravascular lymphoma. Ann Oncol 2004; 15: 1215-1221.

[22] Shimada K, Matsue K, Yamamoto K, Murase T, Ichikawa N, Okamoto M, Niitsu N, Kosugi H, Tsukamoto N, Miwa H, Asaoku H, Kikuchi A, Matsumoto M, Saburi Y, Masaki Y, Yamaguchi M, Nakamura S, Naoe T and Kinoshita T. Ret-

rospective analysis of intravascular large B-cell lymphoma treated with rituximab-containing chemotherapy as reported by the IVL study group in Japan. J Clin Oncol 2008; 26: 3189-3195.
[23] Rosner M, Siegel N, Valli A, Fuchs C and Hengstschlager M. mTOR phosphorylated at S2448 binds to raptor and rictor. Amino Acids 2010; 38: 223-228.
[24] Rosner M and Hengstschlager M. Cytoplasmic and nuclear distribution of the protein complexes mTORC1 and mTORC2: rapamycin triggers dephosphorylation and delocalization of the mTORC2 components rictor and sin1. Hum Mol Genet 2008; 17: 2934-2948.
[25] Drakos E, Rassidakis GZ and Medeiros LJ. Mammalian target of rapamycin (mTOR) pathway signalling in lymphomas. Expert Rev Mol Med 2008; 10: e4.
[26] Wanner K, Hipp S, Oelsner M, Ringshausen I, Bogner C, Peschel C and Decker T. Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. Br J Haematol 2006; 134: 475-484.
[27] Coiffier B and Ribrag V. Exploring mammalian target of rapamycin (mTOR) inhibition for treatment of mantle cell lymphoma and other hematologic malignancies. Leuk Lymphoma 2009; 50: 1916-1930.
[28] Gupta M, Ansell SM, Novak AJ, Kumar S, Kaufmann SH and Witzig TE. Inhibition of histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell lymphoma by inhibiting Akt signaling through mTORC2. Blood 2009; 114: 2926-2935.
[29] Rocca A, Minucci S, Tosti G, Croci D, Contegno F, Ballarini M, Nole F, Munzone E, Salmaggi A, Goldhirsch A, Pelicci PG and Testori A. A phase I-II study of the histone deacetylase inhibitor valproic acid plus chemoimmunotherapy in patients with advanced melanoma. Br J Cancer 2009; 100: 28-36.
[30] Cotto M, Cabanillas F, Tirado M, Garcia MV and Pacheco E. Epigenetic therapy of lymphoma using histone deacetylase inhibitors. Clin Transl Oncol 2010; 12: 401-409.