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Rohan Kodgule

Jie Chen

Pooja Khonde

Joshua Robinson

Amy D'Albora

See next page for additional authors

Authors

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TO THE EDITOR:

Recurrent switch 2 domain *RAC2* mutations in intravascular large B-cell lymphoma

Rohan Kodgule,[1] Jie Chen,[2] Pooja Khonde,[1] Joshua Robinson,[1] Amy D'Albora,[3] Lisa Cook,[3] Catrina C. Fronick,[3] Robert Fulton,[3] Sridhar Nonavinkere Srivatsan,[4] Patrick J. Cimino,[5] and Eric J. Duncavage[1]

[1]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE; [3]McDonnell Genome Institute, Washington University School of Medicine, St. Louis, MO; [4]Division of Oncology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO; and [5]Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA

Intravascular large B-cell lymphoma (IVLBCL) is an extremely rare extranodal B-cell lymphoma characterized by the selective growth of large lymphoma cells inside the lumens of small- and intermediate-sized blood vessels. IVLBCL has an age-adjusted incidence of 0.095 per 1 000 000 and has a propensity to involve the skin and central nervous system.[1] Presenting symptoms in IVLBCL are highly heterogenous and most commonly include cutaneous lesions. However, approximately one-third of patients present with only transient neurologic changes, making IVLBCL challenging to diagnose,[2] and in some patients, a diagnosis may only be confirmed at autopsy. The biology of IVLBCL is not well characterized, and the mechanism behind the unusual localization of lymphoma cells within the lumen of the blood vessels is poorly understood.

Given the rarity of the disease and difficulties obtaining sufficient diagnostic material for study, limited genomic data from IVLBCL is available. Shimada et al[3] demonstrated genomic variants mainly using cell-free DNA obtained from patients with IVLBCL using whole-exome sequencing. Schrader et al[4] and Suehara et al[5] performed targeted sequencing in IVLBCL cases. The mutational spectrum of IVLBCL from these studies shows similarities to the MCD[6,7] subtype of diffuse large B-cell lymphoma (DLBCL), characterized by $MYD88^{L265P}/CD79B$ mutation. MCD subtype is dominated by activated B-cell–type DLBCL and has a significantly worse prognosis.[7] $MYD88^{L265P}/CD79B$ mutation also typically coexists at higher frequency in extranodal DLBCL.[6]

In this study, we identified 6 cases of IVLBCL in autopsy patients seen at Washington University in St. Louis between 1996-2018. The diagnosis of IVLBCL was made antemortem in 1 case and postmortem in the remaining 5. Slides were rereviewed by a boarded hematopathologist (E.J.D.) and 2 neuropathologists (J.C. and P.J.C.) to confirm the diagnosis (Figure 1A-D; supplemental Figure 5). All autopsies were performed under unrestricted permits for research studies. Areas involved by IVLBCL were microdissected using 1 mm core punches of paraffin block with approximate tumor content of >90%. Paired normal tissue was used from 3 patients. Exome libraries were prepared using extracted DNA from involved areas in each case and sequenced on an Illumina NovaSeq 6000. Data were analyzed using the Illumina DRAGEN Bio-IT Platform v3.8 workflow for single-nucleotide variant and indel calling. Variants were annotated using Annovar.[8] Variants were excluded if the variant allele frequency was <2%, total depth was <8×, the variant was not predicted to alter protein coding, or the variant was present in normal populations in >0.2% of individuals (Supplemental Materials). Copy number alterations were analyzed using CNVkit.[9]

Mean exome coverage per patient was 144.9×. The median number of coding variants detected per patient was 195 (supplemental Data). C>T transitions were the most common alteration (supplemental Figures 1-2). All 6 IVLBCL cases had recurrent genetic aberrations that are part of the "MCD type" of
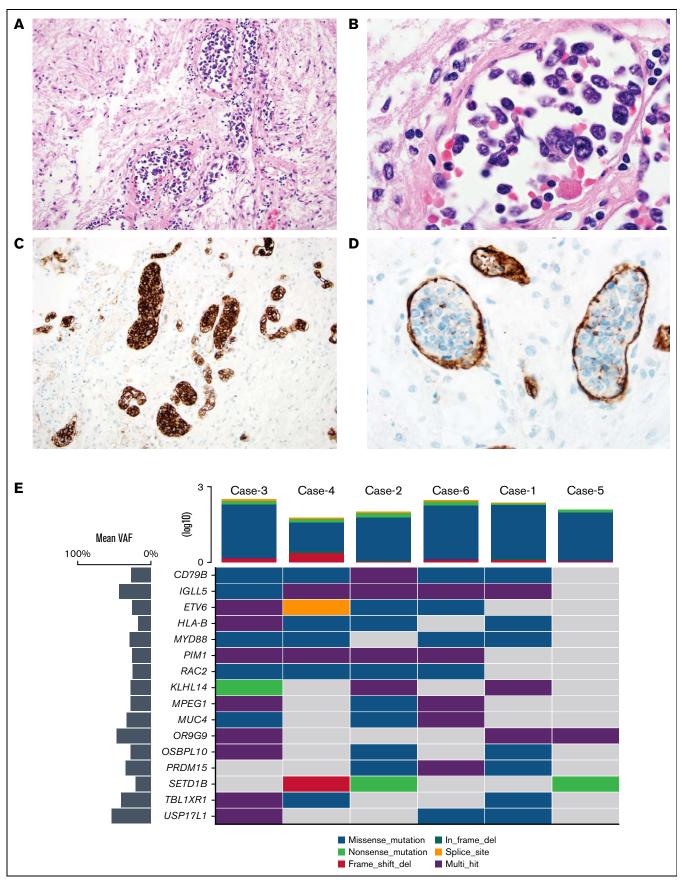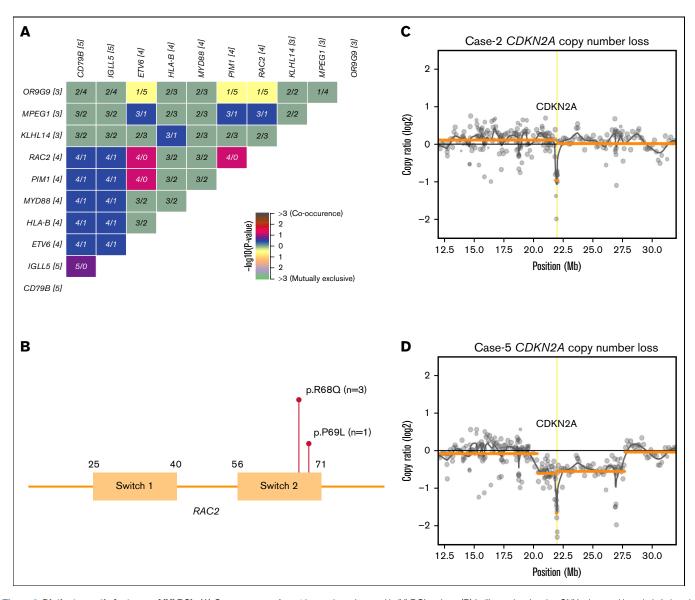
Figure 1.

Figure 2. Distinct genetic features of IVLBCL. (A) Cooccurrence of top 10 mutations detected in IVLBCL cohort. (B) Lollipop plot showing SNVs detected in switch 2 domain of Rho GTPase family member *RAC2* in 4 patients of IVLBCL. (C-D) Copy number loss of *CDKN2A* on chromosome 9p21 in 2 patients detected in our cohort. SNV, Single nucleotide variants.

DLBCL[6] and genes commonly implicated in somatic hyper-mutations in lymphomas[10,11] (Figures 1 and 2A; supplemental Figure 1; supplemental Table 1). Five of 6 cases had *CD79B* (NM_001039933.3) mutations (4 variants at p.Y197 in exon 5 and 1 at p.L200 in exon 6) and had high cooccurrence with *MYD88* p.L265P (n = 4), *RAC2* (n = 4), and genes that are common targets of somatic hypermutations like *IGLL5*, *ETV6*, *HLA-B*, and *PIM1*[3,11] (Figure 2A; supplemental Figure 3).

RAC2 mutations were detected in 4 patients (66%) in the highly conserved switch 2 domain[12,13] (Figure 2B). Ras-related C3 botulinum toxin substrate 2 (*RAC2*) is a member of the ρ family GTPases involved in several cellular functions.[14-16] *RAC2* is specifically expressed in hematopoietic lineage and regulates different cytoskeletal responses and homotypic adhesion in B cells.[15,17] A similar pattern of mutations in the *RAC2* gene was previously reported in cell-free DNA in only 5 cases (23.8%) within 21

Figure 1. Histopathology and recurrent SNVs in IVLBCL. (A) H&E-stained section of the pituitary from case no. 4, which had a *RAC2* switch 2 domain mutation p. R68Q (NM 002872.5) (×20 original magnification shows large, atypical lymphocytes within sized vessels). (B) High-power H&E image showing large, atypical lymphocytes within the vascular lumen (×100 original magnification). (C) A CD20 immunostain demonstrates large intravascular B cells (×20 original magnification). (D) A CD34 immunostain confirms that large cells are confined to the vascular lumen (×60 original magnification). (E) Oncoplot[24] showing top cooccurring mutations detected in 6 patients with IVLBCL. Horizontal lines represent genes, and the vertical line indicates a single patient. The type of mutations is color-coded in the legend. Mean variant allele frequency for each gene is depicted in the bar plot on the left-hand side. The tumor mutation burden (TMB) for each patient is represented on the upper side of the plot. H&E, hematoxylin and eosin.

patients with IVLBCL.[3] Two large studies in primary DLBCL not otherwise specified (NOS) by Schmitz et al[7] (n = 574) and Chapuy et al[18] (n = 304) had only 10 cases with *RAC2* mutations, of which, 4 cases had mutations in the switch 2 domain, which is between amino acid 56 to 71.[19] Mutation in the switch 2 domain of *RAC2* (p.E62K) results in sustained active guanosine triphosphate (GTP) bound *RAC2*[13,14] (supplemental Figure 4). *RAC2* is activated on B-cell receptor (BCR) stimulation and acts as a link between BCR activation to cell adhesion via its physical association with B-cell linker protein.[20] *RAC2* plays an important role in the adhesion of B cells to intracellular adhesion molecule-1 (ICAM-1), and constitutively active *RAC2* creates more adherent B cells.[16] In mice, *RAC2* is one of the key genes responsible for lymphoma progression.[21]

Three cases in our cohort had a mutation in the tumor suppressor *KLHL14*, which has been previously reported in IVLBCL cases by Shimada et al.[3] *KLHL14* inactivation in MCD cells maintains NF-κB signaling even in the presence of ibrutinib.[22] Interestingly, a genome-wide CRISPR/Cas9 screen comparing effects of *KLHL14* knockout on ibrutinib response in MCD-type DLBCL cell line TMD8 revealed *RAC2* among the top 10 genes that sensitized to ibrutinib to a greater extent in wild-type *KLHL14*,[22] and thus, the loss of *KLHL14* might be used to predict ibrutinib treatment resistance in such cases. *CDKN2A* deletion at 9p21 is commonly seen in activated B-cell DLBCL and is associated with a poor prognosis.[23] This deletion was present in ~86% of the IVLBCL cases in a study by Shimada et al using cell-free DNA.[3] In our cohort, we detected 2 out of 6 cases with *CDKN2A* deletion (Figure 2C-D)

Given the low incidence of IVLBCL and difficulty in establishing the diagnosis, we conducted a histologic review of sampled organs to determine the most frequent sites of involvement. Only 1 of the patients in our series had cutaneous disease documented before or at autopsy. Most frequently involved sites included the liver, heart, and kidney (5 of 6 cases). Central nervous system and pituitary were involved in 3 whereas spleen, lungs, and adrenals were involved in 2 of 6 cases. Organs with a low incidence of involvement included testes, prostate, and uterus (supplemental Data).

To our knowledge, this is the first study to evaluate histologically confirmed IVBCL tissue by exome sequencing as opposed to surrogate DNA sources such as cell-free DNA. Given the sparse nature of involvement in most IVBCL biopsies, the use of postmortem tissue allowed for wide sampling of involved areas that would be difficult to evaluate by antemortem biopsy. The study also demonstrates the continued utility of hospital-based autopsies in the genomic era. Using exome data obtained from 6 postmortem patients with IVLBCL, we demonstrate that the mutation spectrum of IVLBCL is most like the MCD type of DLBCL, characterized by gain-of-function mutations targeting *CD79B* BCR subunit and Toll-like receptor signaling adaptor *MYD88*. Recurrent mutations in the switch 2 domain of *RAC2,* which are rare in DLBCL not otherwise specified (NOS), were present in 4 of 6 patients in our cohort. The lower frequency of *RAC2* mutations in IVLBCL cases reported by Shimada et al[3] may be due to the use of surrogate circulating tumor DNA as opposed to actual tumor tissue used in this study. We hypothesize that activating *RAC2* mutations in the background

of chronic BCR signaling may lead to increased adhesion and sustained growth of B cells inside the lumen of the blood vessels. Targeted panels analyzing IVLBCL should include *RAC2* gene in addition to the existing known recurrent mutations. Further studies are required to determine the role of *RAC2* mutations in a chronic activated-BCR signaling state and the dynamics of interaction with the vascular lumen.

ORCID profiles: R.K., 0000-0002-1711-8641; J.C., 0000-0001-6280-5320; C.C.F., 0000-0002-2766-9551; S.N.S., 0000-0001-6293-4632; P.J.C., 0000-0003-0441-4502; E.J.D., 0000-0003-4199-8906.

Correspondence: Eric J. Duncavage, Department of Pathology and Immunology, Washington University in St. Louis, 660 Euclid Ave #8118, St. Louis, MO 63105; email: duncavagee@wustl.edu.

References

1. Rajyaguru DJ, Bhaskar C, Borgert AJ, Smith A, Parsons B. Intravascular large B-cell lymphoma in the United States (US): a population-based study using Surveillance, Epidemiology, and End Results program and National Cancer Database. *Leuk Lymphoma*. 2017;58(9):1-9.

2. Ponzoni M, Campo E, Nakamura S. Intravascular large B-cell lymphoma: a chameleon with multiple faces and many masks. *Blood*. 2018;132(15): 1561-1567.

3. Shimada K, Yoshida K, Suzuki Y, et al. Frequent genetic alterations in immune checkpoint-related genes in intravascular large B-cell lymphoma. *Blood*. 2021; 137(11):1491-1502.

4. Schrader AMR, Jansen PM, Willemze R, et al. High prevalence of *MYD88* and *CD79B* mutations in intravascular large B-cell lymphoma. *Blood*. 2018;131(18): 2086-2089.

5. Suehara Y, Sakata-Yanagimoto M, Hattori K, et al. Liquid biopsy for the identification of intravascular large B-cell lymphoma. *Haematologica*. 2018; 103(6):e241-e244.

6. Wright GW, Huang DW, Phelan JD, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell*. 2020;37(4):551-568.e14.

7. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.

8. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010; 38(16):e164.

9. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. *PLOS Comput Biol.* 2016;12(4):e1004873.

10. Krysiak K, Gomez F, White BS, et al. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma. *Blood.* 2017; 129(4):473-483.

11. Alkodsi A, Cervera A, Zhang K, et al. Distinct subtypes of diffuse large B-cell lymphoma defined by hypermutated genes. *Leukemia.* 2019;33(11):2662-2672.

12. Gasper R, Thomas C, Ahmadian MR, Wittinghofer A. The role of the conserved switch II glutamate in guanine nucleotide exchange factor-mediated nucleotide exchange of GTP-binding proteins. *J Mol Biol.* 2008;379(1):51-63.

13. Hsu AP, Donkó A, Arrington ME, et al. Dominant activating RAC2 mutation with lymphopenia, immunodeficiency, and cytoskeletal defects. *Blood.* 2019; 133(18):1977-1988.

14. Arrington ME, Temple B, Schaefer A, Campbell SL. The molecular basis for immune dysregulation by the hyperactivated E62K mutant of the GTPase RAC2. *J Biol Chem.* 2020;295(34):12130-12142.

15. Croker BA, Tarlinton DM, Cluse LA, et al. The Rac2 guanosine triphosphatase regulates B lymphocyte antigen receptor responses and chemotaxis and is required for establishment of B-1a and marginal zone B lymphocytes. *J Immunol.* 2002;168(7):3376-3386.

16. Arana E, Vehlow A, Harwood NE, et al. Activation of the small GTPase Rac2 via the B cell receptor regulates B cell adhesion and immunological-synapse formation. *Immunity.* 2008;28(1):88-99.

17. Gerasimčik N, He M, Dahlberg CIM, Kuznetsov NV, Severinson E, Westerberg LS. The small Rho GTPases Rac1 and Rac2 are important for T-cell independent antigen responses and for suppressing switching to IgG2b in mice. *Front Immunol.* 2017;8:1264.

18. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes [published corrections appear in *Nat Med.* 2018;24(8):1290-1291 and 2018;24(8):1292]. *Nat Med.* 2018;24(5):679-690.

19. Bunney TD, Opaleye O, Roe SM, et al. Structural insights into formation of an active signaling complex between Rac and phospholipase C gamma 2. *Mol Cell.* 2009;34(2):223-233.

20. Wu W, Wang W, Franzen CA, et al. Inhibition of B-cell receptor signaling disrupts cell adhesion in mantle cell lymphoma via RAC2. *Blood Adv.* 2021;5(1): 185-197.

21. Meacham CE, Ho EE, Dubrovsky E, Gertler FB, Hemann MT. In vivo RNAi screening identifies regulators of actin dynamics as key determinants of lymphoma progression. *Nat Genet.* 2009;41(10):1133-1137.

22. Choi J, Phelan JD, Wright GW, et al. Regulation of B cell receptor-dependent NF-κB signaling by the tumor suppressor KLHL14. *Proc Natl Acad Sci USA.* 2020;117(11):6092-6102.

23. Jardin F, Jais J-P, Molina T-J, et al. Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: a GELA study. *Blood.* 2010;116(7): 1092-1104.

24. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res.* 2018; 28(11):1747-1756.