To the editor:

Differential gene expression in central nervous system lymphoma

While primary central nervous system lymphoma (PCNSL) may be classified among the 3 established molecular subclasses of large B-cell lymphoma: germinal center, activated B-cell, and type 3 based upon gene expression profiles,[1] 2 recent microarray-based expression profiling studies, one performed by our group[1] and another by Tun et al,[2] identified molecular characteristics that distinguish CNS lymphomas from nodal and/or extranodal large B-cell lymphoma. Using a cDNA-microarray–based platform, we identified more than 400 clones that distinguished 23 cases of PCNSL from 9 cases of nodal large B-cell lymphoma. Only a subset (29) of these clones were identified in our report. Using an oligonucleotide-based platform, Tun et al identified more than 60 genes that distinguished 13 cases of PCNSL from 11 nodal and 19 extranodal large B-cell lymphomas.[2]

Determination of the molecular features of CNS lymphoma is significantly more challenging than for systemic non-Hodgkin lymphoma. The architecture of CNS lymphoma is often heterogeneous, with variable cell density and neovascularization, and infiltration by immune effector cells.[1,3,4] The molecular profiling of individual small brain biopsy specimens may, therefore, provide an incomplete analysis of the tumor and its microenvironment. We proposed that there are at least 2 major growth patterns of PCNSL as evidenced by the histopathology of diagnostic tumor specimens: approximately one-half of cases of PCNSL are of low tumor cellularity in which normal brain elements are evident within the neoplasm; the remainder of tumors are of high cellular density without intervening normal brain elements between the neoplastic cells (Figure 1). High-density tumors that express activated STAT-6 are associated with a worse prognosis after treatment with standard methotrexate-based regimens than tumors of low cell density, independent of STAT-6 activation status.[1]

Neurons and glia are a rich source of molecules involved in signal transduction, including proto-oncogenes such as bcl-2.[5-7] Therefore, molecular profiling of PCNSL specimens of low tumor density would clearly be prone to sampling error, given the presence of these interspersed normal brain elements. Given that both our study and that of Tun et al profiled specimens of dense tumor cellularity, it follows that these studies may be biased toward the most aggressive cases of PCNSL. We believe this issue has not previously been discussed in the literature.

Finally, the significance of these studies is limited because each lacks an independent validation set and used distinct microarray platforms. Therefore, we believe it to be essential to identify the subset of overlapping genes that are concordant in distinguishing CNS from non-CNS large B-cell lymphoma in each study, especially since only a subset of our data were presented in our publication (Table 1).[1] The evidence for a unique CNS lymphoma molecular signature is underscored by the fact that, while all of the non-CNS DLBCL cases profiled in our study were obtained from lymph node biopsies, 19 of 30 (63%) of the non-CNS lymphoma cases profiled by Tun et al were extranodal in origin and from a variety of organ sites (eg, tonsil, bone, testes). This heterogeneity likely contributes to the discordance in differentially expressed genes reported in these studies.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

Contribution: J.L.R., P.T., and M.A.S. designed research. J.L.R., C.K., A.S., and P.T. performed research. T.T.B. provided specimens for the research. J.L.R., C.K., A.S., P.T., and M.A.S. analyzed and interpreted data. J.L.R., C.K., P.T., T.T.B., and M.A.S. drafted the paper.

This work was supported by a National Cancer Institute Research Career Award, University of California San Francisco Brain Tumor Specialized Program of Research Excellence (SPORE), and by grants from Gabrielle's Angel Foundation for Cancer Research (New York, NY) and the American Cancer Society (J.L.R.).

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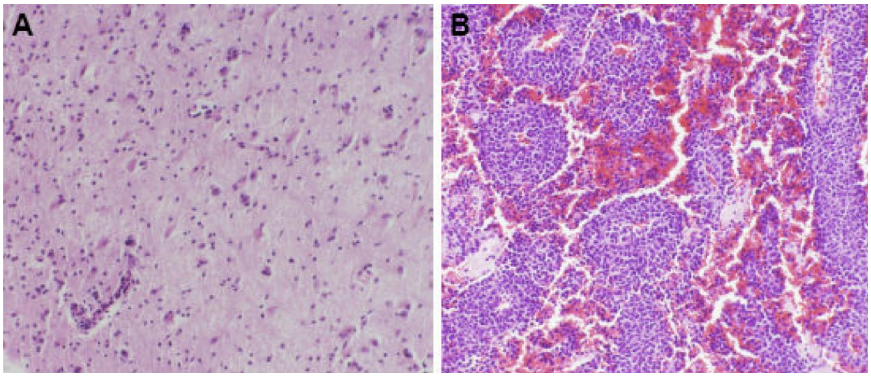

Figure 1. Low- and high-density PCNSL tumors. Low-density (A) and high-density (B) PCNSL tumors, each of large B-cell histology, are distinguished by the presence (A) or absence (B) of intervening normal brain elements between the tumor cells in pathologic specimens. Magnification ×100 (hematoxylin and eosin). Image were photographed using an Olympus Vanox AHBT3 microscope with Olympus DP70 digital camera (Olympus Optical, Tokyo, Japan). Images were captured onto a personal computer using Olympus DP controller software, version 1.2.1.108.

Table 1. Differentially expressed genes in the 2 studies

CNS to non-CNS	Symbol	Rubenstein study		Tun study	
		Fold change	*P*	Fold change	*P*
Up-regulated					
Osteopontin	SPP1	11.4	8.00×10^{-6}	9.73	3.03×10^{-8}
Complement component 1, q, subcomponent	C1QB	2.8	8.29×10^{-6}	2	2.40×10^{-2}
Hemoglobin, alpha2	HBA2	2	6.26×10^{-4}	2.5	1.90×10^{-2}
Regulator of G-protein signaling 13	RGS13	2.3	3.34×10^{-2}	2.4	2.70×10^{-2}
Chitinase 3-like 1	CH13L1	2.8	5.02×10^{-2}	2.72	5.10×10^{-5}
VT-cell leukemia/lymphoma 1A	TCL1A	2.8	1.07×10^{-1}	2.96	5.55×10^{-5}
Down-regulated					
Nicotinamide N-methyltransferase	NNMT	0.56	5.26×10^{-3}	0.43	8.99×10^{-4}
Vascular endothelial growth factor C	VEGFS	0.51	7.16×10^{-3}	0.4	1.00×10^{-3}
Collagen, type VI. alpha 1	COL6A1	0.45	1.32×10^{-2}	0.45	3.25×10^{-4}
Latexin	LXN	0.76	1.99×10^{-2}	0.48	2.67×10^{-2}
Lumican	LUM	0.5	2.27×10^{-2}	0.28	8.45×10^{-3}
Laminin, alpha 4	LAMA4	0.74	4.54×10^{-2}	0.37	8.00×10^{-3}

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Response:

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Our group published a microarray study of primary central nervous system lymphoma (PCNSL) in *Blood* in March 2008.[1] We reported a comprehensive CNS signature of PCNSL, identifying single-gene differential expression as well as a pathway signature. Most notably, our pathway signature for PCNSL is characterized by differential expression of extracellular matrix (ECM)- and adhesion-related pathways. The most up-regulated gene is the ECM- and adhesion-related osteopontin (SPP1). Our study is unique in that we compared PCNSL to a broad spectrum of non-CNS diffuse large B-cell lymphoma (DLBCL), consisting of nodal and extranodal samples; and in-depth bioinformatics analysis was performed. This is in contrast to 2 other microarray studies in PCNSL[2,3] in which the comparisons were made to nodal DLBCL and no pathway analysis was performed.

We are pleased that Rubenstein et al have confirmed our single-gene expression findings by retrospective analysis of their data and come up with concordant genes. We are also surprised, because their original findings[2] published in 2006 were very different from ours.[1] We would recommend that they also consider performing pathway analysis on their data. In our opinion, the pathway analysis makes it possible to obtain meaningful biologic insights with gene expression data. They mentioned lack of an independent validation set in both studies. We would point out that in our immunohistochemical validation, 10 of 15 PCNSL samples were not used in the microarray study.[1] As such, we did validation on a largely independent sample set.

Their proposal on 2 major types of PCNSL based on cellular density of the tumor will need more validation. Autopsy studies have shown the presence of lymphoma cells throughout the brain, even in areas that look normal on imaging scans[4,5]; hence the term whole brain disease.[5] One has to assume that high-density and low-density lesions do coexist and that tumors are scattered with intervening normal brain.

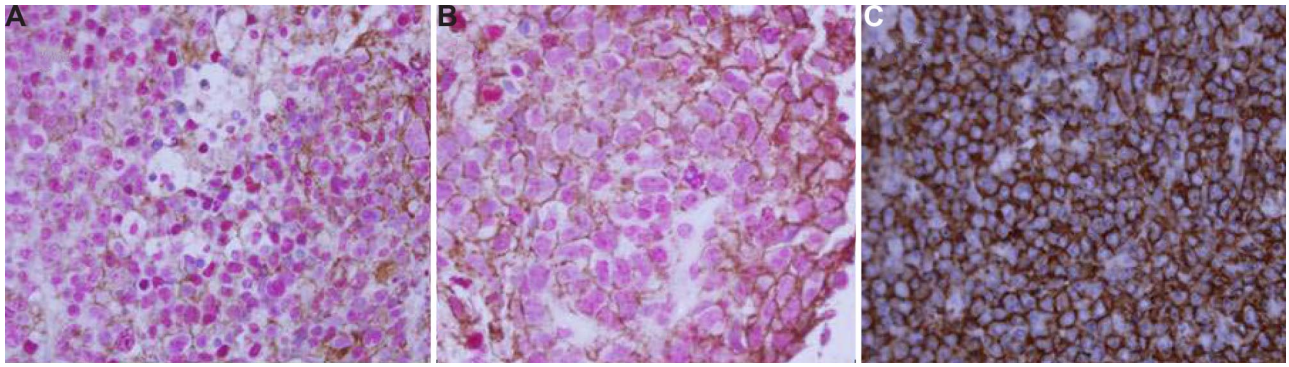

Figure 1. CD20 and osteopontin are coexpressed in PCNSL. Dual immunohistochemistry was performed using antibodies specific to CD20 (Dako, Carpenteria, CA) and osteopontin (R&D Systems, Minneapolis, MN). Diaminobenzidine (brown) dye (Dako) was used for CD20 and Vulcan fast red (red) dye (Biocare Medical, Concord, CA) was used for osteopontin. (A) PCNSL. Original magnification ×400. Both CD20 (membranous pattern) and osteopontin (predominantly nuclear) are clearly positive. (B) Second case of PCNSL. Original magnification ×1000. Again, both CD20 and osteopontin are positive. (C) Nodal DLBCL. Original magnification ×400. Only CD20 is positive with no expression of osteopontin. A Leica DMLB optical microscope (Leica Microsystems, Wetzlar, Germany) and Cytoseal-60 mounting media (Richard Allen, Kalamazoo, MI) were used. Images were acquired using a SPOT RT Color Camera (Diagnostic Instruments, Sterling Heights, MI), and were processed with SPOT Advanced program version 2.0 (Diagnostic Instruments) and Adobe Photoshop version 6.0 software (Adobe Systems, San Jose, CA).