Proteomic characterization of primary diffuse large B-cell lymphomas in the central nervous system

Laboratory investigation

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Object. The lack of primary lymphoid tissue within the central nervous system (CNS) confounds our understanding of the pathogenesis of primary CNS lymphomas (PCNSLs). Comparing the protein expression of PCNSLs and sporadic systemic lymphomas (SSLs) provides a useful strategy for identifying a molecular signature that characterizes disease-associated features and provides information regarding tumor initiation and progression.

Methods. Seven diffuse large B-cell PCNSLs were selected to undergo 2D gel electrophoresis, and profiled proteomes from these PCNSLs were compared with those from 7 diffuse large B-cell SSLs. Distinguishing proteins were sequenced using mass spectrometry.

Results. Two-dimensional gel electrophoresis identified an average of 706 proteins from each specimen. Computerized gel analysis and manual reconfirmation revealed a 96% similarity in the proteomes of PCNSLs and SSLs. Comparative analysis identified 9 proteins significantly overexpressed (p < 0.05) and 16 proteins downregulated in PCNSLs. The proteomic findings were further validated using Western blot and immunohistochemical staining.

Conclusions. The similarities in proteomic patterns between PCNSLs and SSLs suggest that these tumor types share structural similarities, acquired during differentiation. The ultimate fate of lymphomatous cells (CNS vs systemic) may be related to differentially expressed proteins, which function in homing and host processing. Elucidating the roles of these differentially expressed proteins will prove valuable in understanding the pathogenesis of PCNSL.

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Key Words • B-cell lymphoma • gel electrophoresis • proteomics • sporadic systemic lymphoma • Western blot

Primary CNS lymphomas are extranodal malignant lymphomas arising in the CNS in the absence of detectable disease outside the CNS at the time of diagnosis.⁶ Despite the fact that PCNSLs account for 0.8–6.6% of primary brain tumors, the relationship between PCNSLs and SSLs is unknown. Extraneural large B-cell lymphomas are mature B-cell neoplasms that originate from the secondary germinal centers or postgerminatal centers of lymphoid tissue.⁷ However, the CNS lacks a lymphatic system or a secondary germinal center/postgerminal center–like structure, and it is thus unknown whether PCNSLs share a common lineage with their extraneuronal counterparts or comprise a distinct disease entity.

Comparative analyses between PCNSLs and SSLs have contributed to the understanding of the origin of PCNSLs. Both tumor subclasses are indistinguishable on histological examination when they are of the same pathological subtype. Furthermore, surface markers for identifying subtypes of PCNSL and SSL, such as Bcl-6 and CD10 for mature B-cell type lymphomas, are identical.¹²,⁴⁵,⁵⁰,⁶⁶ The advent of high-resolution genomic and molecular techniques including microarray gene expression profiling and the array comparative genomic hybridization have further demonstrated similarities between...
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PCNSLs and SSLs, as well as unique genes that appear to be expressed in PCNSLs, and possibly relate to the tumor’s ability to migrate and survive in the CNS milieu. To further elucidate the relationship between these tumor types, it is necessary to understand differences in their protein expression. This differential expression likely relates to the tumors’ affinity for the CNS or periphery, as well as their ability to migrate to the CNS.

There has been a limited amount of work performed on protein expression in lymphomas. Nozaki and colleagues detected a set of proteins that were distinctly expressed in PCNSLs by immunohistochemical examination, and concluded that PCNSLs in immunocompetent patients has a differential expression of p53, Bcl-2, Bcl-6, c-myc, and Mdm-2, indicating that a PCNSL in an immunocompetent host is a disease distinct from an SSL. Mielini and associates demonstrated that protein expression in the cerebrospinal fluid may relate to CNS involvement in lymphomas. However, reports such as these focus on only a small fraction of well-known tumorigenic factors, and there are likely other proteins expressed that play a major role in PCNSL development.

Proteomics is the study of the expressed protein complement in a biological tissue or fluid (also known as the proteome). The proteome, transcriptome, and genome are highly complementary systems, and correspond, respectively, to the protein, mRNA, and DNA in a cell, tissue, or organ. Consequently, the proteome of human lymphomas likely represents the key determinants of their structure and function and may provide information on the molecular alterations that underlie the differences in pathogenesis between the CNS and peripheral tumor subtypes.

Proteomic analysis provides information on the major differences between PCNSLs and SSLs as well as clues that could be valuable in understanding tumorigenesis of PCNSLs. This study focuses on the comparative proteomic analysis of PCNSLs and SSLs.

**Methods**

**Patients and Clinical Data**

All specimens were diffuse large B-cell lymphomas (PCNSL-B or SSL-B). Seven frozen PCNSLs from 3 males and 4 females (mean age 67 years, range 59–73 years) and 7 frozen SSLs (all nodal) from 3 males and 4 females (mean age 70 years, range 53–81 years) were collected at the Division of Hematology in the Department of Neurosurgery, Niigata University, Niigata, Japan. Six paraffin-fixed PCNSLs from 3 males and 3 females (mean age 55 years, range 49–65 years) and 5 SSLs from 2 males and 3 females (mean age 57 years, range 50–62 years; 4 nodal and 1 testicular SSL-B) were collected at the Department of Neuropathology and Ophthalmic Pathology, Armed Forces Institute of Pathology, Washington, DC. Tissues and clinical information were obtained as part of a study approved by the institutional review boards at the institutes noted above. Tumors were reviewed by 2 independent pathologists and classified according to the World Health Organization histological classification. All patients were negative for the Epstein–Barr virus and were immunocompetent.

**Selective Tissue Dissection and Protein Preparation**

To avoid procurement of normal tissue or areas of necrosis, hemorrhage, and inflammation, selective tissue dissection was performed prior to protein analysis as previously described. Tissues for Western blot analysis were homogenized in T-PER Tissue Protein Extraction Reagent (Pierce, Thermo Fisher Scientific) with a protease inhibitor combination (Roche Molecular Biochemicals) and the supernatants were used after centrifugation. The tissues for 2D gel electrophoresis were dissolved into extraction buffer II containing 8 M urea, 4% (w/v) Bio-Lyte 4/7, and 2 mM tributyl phosphate (Bio-Rad), vigorously mixed in a vortex, and centrifuged. The average protein concentration was 70 µg per sample. The supernatant was combined with a rehydration buffer mixture containing Rehydration Buffer (8 M urea, 2% 3-[3-cholamidopropyl] dimethylammonio]-1-propane sulfonate, 50 mM dithiothreitol, and 0.2% [w/v] Bio-Lyte 4/7 ampholytes; Bio-Rad), immobilized pH gradient buffer (Amersham Biosciences), and bromophenol blue, and subsequently rehydrated overnight using Immobiline DryStrips (pH 4–7, 11 cm; Amersham Biosciences) on a Reswelling Tray (Amersham Biosciences).

**Two-Dimensional Gel Electrophoresis**

The isometric focusing for the first dimensional electrophoresis was performed using a MultiPhore II Electrophoresis System (Amersham Biosciences). The strips were subjected to voltages of 300–3500 V. Immobilized pH gradient strips were equilibrated using Equilibration Buffer I containing 6 M urea, 2% SDS, 375 mM Tris-HCL (pH 8.8), 20% glycerol, and 2% (w/v) dithiothreitol; and Buffer II containing 6 M urea, 2% SDS, 375 mM Tris-HCL (pH 8.8), 20% glycerol, and 2.5% (w/v) iodoacetamide (Bio-Rad). Precast ExcelGel SDS gels (12–14% Gradient gel, pH 4–7, 245 × 180 × 0.5 mm; Amersham Biosciences) were used for the 2D protein separation by a MultiPhore II Flatbed System (Amersham Biosciences) under a constant voltage of 700 V. A silver staining kit (Amersham Biosciences) was used to detect protein spots according to the manufacturer’s instructions. All samples were processed in duplicate.

**Imaging and Statistical Analysis**

Digital images were acquired for each sample gel and the optical density percentage of the spots was assumed to be proportional to the protein concentration. The protein spots were detected, quantified, and matched using Proteomweaver software (Definiens). The images were also manually analyzed to avoid improper image alignment from the computerized program. Individual spot volumes were normalized against total spot volumes for a given gel. Differences in apparent protein expression levels between the PCNSL-Bs and SSL-Bs were considered potentially significant when matched spots exhibited at least a statistical difference (p < 0.05) in their averaged optical density percentage, whereas protein spots without optical density percentage difference (p > 0.05) were counted as “identical” proteins between groups. Because the 2 groups were independent entities, and their respec-
BioBasic-18 column (0.18 mm, ThermoElectron) operated in the high-throughput mode. MS on a ProteomeX liquid chromatography/MS system digestions were analyzed by liquid chromatography MS/MS eluted at 1–2 µl/min with a gradient of 2–50% mobile phase B over 30 minutes. Mobile phase A was H2O (0.1% formic acid) and mobile phase B was CH3CN (0.1% formic acid). Column effluent was analyzed on the LCQ Deca XP Plus (ThermoElectron) operating in the “Top Five” mode. Uninterpreted MS/MS spectra were searched against a human database utilizing the BioWorks and SEQUEST programs (ThermoElectron). A protein identification was accepted when MS/MS spectra of ≥ 2 peptides from the same protein exhibited at a minimum the default cross correlation coefficient score versus charge values set by the program (for Z = 1, 1.50; for Z = 2, 2.00; and for Z = 3, 2.50).

**Western Blot**

Forty micrograms of each tissue lysate was loaded on to 4–20% SDS–polyacrylamide gel (Invitrogen). Proteins were electrophoretically transferred to nitrocellulose membranes (Invitrogen), blocked using Starting-Block Blocking Buffer (Pierce), washed, and incubated with primary antibodies (1:200 dilution for all antibodies), including polyclonal antibodies against PDI (Santa Cruz Biotechnology, Inc.), monoclonal antibodies against GFAP (Chemicon International), monoclonal antibodies against ANX5 (Neomarkers), and polyclonal antibodies against HSP27 (Neomarkers), and polyclonal antibodies against ANX5 (Santa Cruz Biotechnology, Inc). Beta-actin antibody (1:500, Sigma) was used as an internal control. Secondary antibodies were purchased from Santa Cruz Biotechnology, Inc., and appropriately applied to individual blots according to the species origin of the applied primary antibodies. Signals were detected by Enhanced Chemiluminescence Substrate (Pierce).

**Immunohistochemical Analysis**

To investigate the expression of selected proteins in situ, specimens were subjected to immunohistochemical analysis using primary antibodies (1:100 dilutions for all antibodies) against PDI, ANX5, and HSP27. Briefly, antigen retrieval was applied to the paraffin-fixed slides and the primary antibody incubation was performed for 3 hours at room temperature. The immunosignal was generally visualized by staining with 3′,3-diaminobenzidine solution (dark brown, Vector Laboratories, Inc.), which was catalyzed by biotinylated horseradish peroxidase. To investigate the coexpression of GFAP and CD44 (hom-ing-associated cell adhesion molecule) in glial cells of PCNSLs, double-immunohistochemical/immunofluorescent staining was performed; in the immunohistochemical staining, CD44 (1:100 dilution for its antibody, Santa Cruz Biotechnology, Inc.) was stained with 3′,3-diaminobenzidine and GFAP was subsequently stained with Vector-Red (Vector Laboratories, Inc.), in the immunofluorescent staining, CD44 was stained red using Rhodamine Red (Jackson ImmunoResearch Laboratories, Inc.), GFAP was stained with green using fluorescein isothiocyanate (Jackson ImmunoResearch Laboratories, Inc.), and nuclei were stained blue using 4,6-diamino-2-phenylindole-dihydrochloride (Vector Laboratories, Inc.).

**Results**

**Two-Dimensional Gel Electrophoresis**

The 2D gel electrophoresis was performed in duplicate, providing a reproducible proteomic pattern for each sample. Silver staining detected > 700 spots on gels within a pH range of 4 to 7 and a molecular mass range from 10 to 250 kD (Fig. 1). Gels from PCNSLs were matched, normalized, and compared with SSL gels. Proteins that consistently appeared in PCNSLs and were absent in SSLs were recorded. Proteins unique to the SSLs were analyzed using the same method. Next, using computerized and manual analyses, the proteomic patterns between the 2 groups were compared with that of other primary brain tumors and lymphomas from other differentiation stages (Appendix, Fig. 1). Nine proteins were markedly upregulated and 16 were downregulated in PCNSLs compared with SSLs. Overall, only ~ 4% of the proteins (25 of the average 706 protein spots on 2D gel electrophoresis) were differentially expressed between PCNSLs and SSLs in this study.

**Protein Identification**

From the 2D gel electrophoresis comparison, these 25 distinguishing proteins were selected for MS sequencing. The proteins were identified from 2 or more unique peptide sequences. Compared with SSL proteomes, PCNSLs overexpressed the following 9 proteins: PDI, GFAP, HSP70 protein 5, dimethylarginine dimethylaminohydrolase 1, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, apolipoprotein A-I precursor, immunoglobulin kappa light chain, and 2 other immunoglobulin light chain fragments (Table 1).

We categorized the 16 proteins downregulated in PCNSLs into the following groups: energy metabolism (phosphoglycerate kinase 1, ubiquinol-cytochrome c reductase core protein 1, 6-phosphogluconolactonase, mitochondrial short-chain enoyl-coenzyme A hydratase 1 precursor, and enolase 1 protein); oxidative resistance (HSP27, glutathione peroxidase 1, and DJ-1 protein [Parkinson disease protein 7]); nucleotide synthesis (cytidylyl kinase, endothelial cell growth factor-1 protein); multifunction (ANX5, glia maturation factor gamma, and heme binding protein-1); and 3 immunoglobulin light chain fragments (Table 1). The names, abbreviated symbols, protein database identification numbers, coding
gene locus, and potential functions of these distinguishing proteins are also listed in Table 1.

**Western Blotting**

Western blotting was performed using specific antibodies raised against 4 selected proteins. Western blotting was performed to ascertain the validity of our proteomic findings, with the selection of candidates based on our interests, the availability of antibodies, and the availability of sample specimens left from proteomic analyses. Protein disulfide isomerase and GFAP represent PCNSL-specific proteins, whereas ANX5 and HSP27 represent PCNSL downregulated proteins. The Western blot analysis results confirmed our previous proteomic observations. As shown in Fig. 2, GFAP and PDI were overexpressed in PCNDL-Bs, whereas ANX5 and HSP27 were downregulated.

**Immunohistochemical Evaluation**

Immunohistochemical analysis was performed on paraffin-embedded and frozen tissues, with the same antibodies used for Western blot analysis, which provided additional information about in situ protein expression. As shown in Fig. 3, PDI was localized in the cytoplasm and surface of the PCNSL-Bs, whereas expression of PDI was significantly reduced in SSLs. Annexin 5 was clearly visible in SSLs, and only minimally detected in PCNSL-Bs. Heat shock protein 27 was more abundant in SSLs and was characterized by finely granular cytoplasmic staining. Double staining of the sections of PCNSLs with anti-CD44 and anti-GFAP demonstrated that CD44 and GFAP colocalized to astrocytes. Figure 4A–C summarizes the most prominent features of the distribution of CD44. Perivascular astrocytes surrounding the vessels revealed high levels of expression of CD44 on their surfaces, which was verified by positive binding to anti-GFAP antibodies as indicated by the abundant cytoplasm extending into processes. Figure 4D demonstrates negative staining with both antibodies on an SSL specimen. The immunofluorescent staining indicated the colocalization of GFAP and CD44 in the PCNSL (Fig. 4E), single GFAP expression in astroglia of normal brain tissue (Fig. 4F), but completely negative staining for both GFAP and CD44 in the SSL (Fig. 4G).

**Discussion**

**Origins of CNS Lymphoma**

Primary CNS lymphoma tumors can be grouped into 4 categories: 1) high-grade B-cell lymphomas, 2) low-grade B-cell lymphomas, 3) Burkitt lymphomas; and 4) T-cell lymphomas. Because > 90% of all lymphomas are of the high-grade B-cell type (diffuse large B-cell lymphomas), most of our current knowledge about PCNSLs has arisen from the study of this subtype.41

The B cells arise from the bone marrow and initially migrate to the periphery as naive B cells. They are subsequently transported to lymph nodes and lymphoid follicles of the spleen, where they further mature in germinal centers. Here, they undergo clonal expansion, somatic hypermutation, class-switch recombination, and selection for a functional antigen receptor. Finally, the selected B lymphocytes mature to become activated memory and plasma cells.59

Based on the currently accepted definition of CNS lymphomas, the localization of the initial lesion is critical to differentiate PCNSLs from SSLs. Accordingly, PCNSLs would not be designated “primary” if the CNS were randomly targeted as the homing site by a tumor cell from a sporadic systemic lymphoma or if the CNS was simply an
implantation site from an “invisible” SSL. If PCNSLs do originate independently of SSLs, their likely sources are either from cells conserved within the CNS or from the migration and transformation of nonlymphoma cells.

Peripheral CNS Lymphomas in Germinal or Postgerminal Centers

After they are committed to development, B cells migrate from the bone marrow to peripheral lymphoid
tissue, passing through various stages of differentiation before maturing. A malignancy involving B cells may therefore occur at any developmental stage, resulting in lymphomatous characteristics with conserved stage-specific surface markers, such as Bcl-6, B cell–specific transcriptional coactivator (OCA-B), octamer transcription factor-2 (Oct-2), lymphocyte-specific interferon regulatory factor-4 (MUM/IRF4), paired box-5 (BSP/PAX5), and transcription factor PU 1 (PU1).10,14,16,17,27,32,60,63

Comparison of 2D gel electrophoresis patterns en-

![Image](image1)

**Fig. 2.** Proteomic comparison results were validated by Western blot analysis. Lanes 1–3 represent PCNSLs, and lanes 4–6 represent SSLs. The 4 proteins and their molecular weights are listed on the left. Beta-actin expression is detected as an internal control. This image shows that GFAP and PDI were overexpressed in PCNSL-Bs, whereas ANX5 and HSP27 were downregulated.

![Image](image2)

**Fig. 3.** Immunohistochemical staining on lymphoma tissue validating proteomic results. Panels A, C, and E are PCNSLs; panels B, D, and F are SSLs. Immunohistochemical staining detected PDI expression in PCNLSs (A) at a much reduced level compared with SSLs (B). Annexin 5 expression and HSP27 expression were higher in SSLs (D and F, respectively) but at a minimal level in PCNLSs (C and E, respectively). Original magnification × 400.
ables us to expound on the molecular basis of tumor initiation and differentiation.\textsuperscript{24,34,66} In this study, we demonstrate a high concordance of proteomes (~96%) between PCNSLs and SSLs, indicating highly conserved protein expression. This similarity is much higher than that seen when we compared PCNSLs with other primary brain tumors (such as astrocytomas and meningiomas) in previous reports\textsuperscript{34,47} or when PCNSLs are compared with other types of systemic lymphomas (Appendix, Fig. 1). The common histological characteristics of the 2 tumor types may also stem from these findings.

Our analysis has not identified any differences in stage-specific differentiation markers between PCNSLs and SSLs, suggesting that PCNSLs could originate from the same differentiation stage (germinal center/postgerminal center), given their homology to SSLs. Because germinal centers/postgerminal centers only arise in peripheral lymphatic tissues (tonsil, mucosa-associated lymphatic tissue, and lymph nodes), PCNSLs would have to originate from an extraneuronal germinal center/postgerminal center, and migrate to the CNS by specific homing mechanisms, a mechanism that has been previously suggested.\textsuperscript{13,42,58} This hypothesis could explain the native feature of PCNSLs that appear to exhibit angiotropism in the brain.\textsuperscript{2}

Immunoglobulin Expression as a Clue to PCNSL Development

Somatic hypermutation provides further evidence that lymphoma tumor progenitor cells develop as germinal center or postgerminal center B cells. Evidence for this exists in the fact that lymphomas can have monoclonally rearranged immunoglobulin heavy-chain genes, and exhibit consistent mutations within these rearranged regions.\textsuperscript{42} This feature is characteristic of B cells that have undergone somatic hypermutation, which is carried out in the germinal centers.\textsuperscript{33}

In our analysis, we detected several distinct immunoglobulins in the PCNSL-B and SSL-B proteomes. Although their functional role is still unknown, these immunoglobulins may facilitate the stratification of these 2 unique categories of lymphoma.

Under oncogenic conditions, the presence of intraclonal heterogeneity in immunoglobulin gene mutations
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is regarded as a marker of ongoing somatic mutations that occur almost exclusively in the germinal center and thus is used as a marker of the germinal center region.30,35,36,64 The finding that PCNSL-Bs and SSL-Bs conserve their group-specific immunoglobulins provides supporting evidence for PCNSL-B cells undergoing differentiation within germinal centers, but also suggests the presence of a specialized microenvironment during PCNSL-B development.

Homing Lymphoma Cells to the CNS

Primary CNS lymphomas are unusual in that they are strictly confined to the CNS, an area normally devoid of B cells. The mechanisms involved in this selectivity for the CNS are still unknown; however, 3 hypotheses have been proposed. The first hypothesis is that a patient develops a systemic lymphoma, which infiltrates many organs, including the brain. This lymphoma is nearly eliminated by the immune response during relocalization, however, because the CNS is immunologically privileged, lymphoma in this region escapes destruction. This may be unlikely, due to the fact that other immunologically privileged sites, such as the testis, are usually not involved in PCNSLs.43–45

The second hypothesis is that a normal inflammatory response to trauma or infection in the CNS attracts lymphoid cells. Once in the CNS, they undergo neoplastic change and develop into a lymphoma. However, an increase in the incidence of PCNSLs is not necessarily observed in patients with inflammatory disorders.29 The third hypothesis postulates that the lymphoma develops outside the CNS, yet also develops specific patterns of adhesion molecules that allow it to preferentially target the CNS.29,43–45

Irrespective of which theory is accurate, a key component to the tumorigenesis of PCNSLs is related to the homing process. It is believed that homing to the CNS is either related to lymphomas having a specific capability to grow in the microenvironment of the CNS, due to cellular and molecular factors in the tumor and the CNS milieu, or because of the isolation of the CNS from systemic immune surveillance mechanisms that destroy tumor cells.22,45,54

By comparing the proteomic expression of PCNSL-Bs and SSL-Bs, we are able to identify differences in their proteomic profiles that may help elucidate CNS homing processes. These are subcategorized later on in the Discussion.

Proteins Affecting Molecular Transformation and Immunological Surveillance. Protein disulfide isomerase (the first identified protein) is one of the isolated proteins that may herald molecular transformation of lymphoma cells during their homing process. This multifunctional protein is mainly localized in the endoplasmic reticulum and functions as a catalyst for forming, reducing, or isomerizing disulfide bonds.19,65 Protein disulfide isomerase also remains on the mammalian cell surface; through catalyzing disulfide bonds, this surface-bound PDI can participate in transmembrane signal transductions and membrane receptor modifications.9,15,37,38 The present proteomic comparison indicates a higher expression of PDI in PCNSL-Bs than in SSL-Bs and was verified by Western blot analysis and immunohistochemical staining. These findings suggest that PDI may be involved in a special modification of the surface molecules of the CNS lymphoma cells.

Proteins Affecting Tumor Metabolism. In the early 20th century, Warburg initially identified the fact that tumor cells have an altered glucose metabolism. Since then, it has been hypothesized that alterations in energy metabolism provide growth and survival advantages in certain oncogenic microenvironments.21,25,39,62 In contrast to normal brain cells, in which glycolysis and mitochondrial respiration are tightly coupled, brain tumor cells are generally defective in respiration due to damaged mitochondrial oxidation.38,48,52,57 During the homing process, lymphomatous malignancies might also have modifications of their metabolism to adjust into the CNS microenvironment. Our proteomic analysis demonstrates 2 functional enzymes in mitochondrial oxidation (No. 12 and No. 15) that are downregulated in PCNSL-Bs, representing a possible defect in mitochondrial oxidation. Three energetic metabolism-related proteins (No. 10, No. 14, and No. 22) are also downregulated in PCNSL-Bs. Given the similar metabolic patterns between normal and neoplastic lymphoid cells,50 our findings suggest that PCNSLs may experience specialized modifications in their natural behaviors to facilitate homing and survival within the CNS microenvironment.

Heat Shock Proteins. Heat shock proteins are molecular chaperones involved in many cellular functions,11 including the folding of newly synthesized proteins, protein trafficking across cellular membranes, and the assembly/disassembly of protein complexes. These proteins are differentially expressed in response to various biological and environmental stresses.49 Compared with SSL-Bs, PCNSL-B cells specifically express HSP70 protein, whereas HSP27 expression is maintained at a lower level. Although HSPs are multifunctional proteins and the role of HSPs in PCNSL-B development is still unknown, their alterations in PCNSL-Bs may reflect the phenomenon of self-adjustment that occurs when lymphatic malignancies home to the CNS.

Proteins That Influence the Tumor’s Affinity to the CNS. Adamek and colleagues reported the presence of strongly GFAP-positive astrocytes within the neoplastic infiltrate found along with lymphoma cells, implicating astroglia in the pathophysiology of PCNSLs. We consistently found GFAP in all samples of PCNSL-Bs, which likely represents a hosting response from the local astrocytes during lymphomatous cells homing to the CNS. To prove this, we chose a well-acknowledged homing cell adhesion molecule, also known as CD44, which mediates lymphocyte adhesion to the white matter,2–4,23 along with GFAP for immunostaining our lymphoma tissue. Our data clearly shows that the GFAP-positive cells are perivascular astrocytes from the CNS that intermingle with lymphoma cells in the perivascular area and secrete CD44, which may be related to homing of lymphomatous...
malignancies. The involvement of CD44-positive astrocytes in head injury and tumorigenesis has been well established, and it has been suggested that the production of hyaluronan may contribute to cell adhesion and tumor invasion in this tissue. Figure 4 demonstrates that the most intensive CD44 signal was on GFAP-positive cells that were histologically similar to astrocytes, and we found much less staining or completely negative staining on other cells of PCNSLs and on SSLs.

Proteomic Profiling in Neurosurgery: Advantages and Limitations

The advantages of proteomic profiling include the ability to selectively analyze tumors using tissue microdissection, as well as the sensitivity of silver staining in identifying large numbers of proteins even with a small sample size. The complexity of the CNS arises from a complex array of signal transduction pathways and cellular interaction networks between proteins that influence cellular function. Study of the human genome has provided some insight into these mechanisms; however, this field has limitations because there is no complete correlation between transcriptional profiles and actual protein levels in cells. Ultimately it is important to understand the presence of posttranscriptional and posttranslational modifications, as these modifications provide valuable insights into a disease process and cannot be completely understood using genomics or DNA-microchip analysis.

In a typical proteomic study the investigator is interested in the relative amounts of protein expressed in a tissue with a disease state, or following an intervention compared with tissue that has not been subject to the disease or intervention. The identification of differentially expressed proteins between these two allows the experimenter to understand even small differences between tissues in various pathological conditions.

There are limitations to information revealed by proteomic profiling. Proteomics only reveals a fraction of the proteins found in the human genome, and it is possible that proteins present in low concentrations, or that are expressed outside of the pH range of the 2D gel, are not seen. Furthermore, the volume of tissue obtained for research purposes during a biopsy procedure is often limited, and this limitation is underscored by the fact that tissue is often lost during microdissection and purification. These factors raise the possibility of there being other important structural or functional proteins that will need to be identified using genomics, and other molecular biology techniques in the future.

Conclusions

Using selective tissue dissection and 2D gel electrophoresis/MS/MS, our data indicates a high degree of homology between PCNSLs and SSLs. These proteomic differences likely account, in part, for the differences in biological behavior between the tumors and could play a role in tumorigenesis.

Disclosure

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References


APPENDIX, FIG. 1. Images obtained after 2D gel electrophoresis distinguish PCNSL-Bs and SSL-Bs (C and D, respectively) from primary CNS tumors (astrocytoma, A) and other lymphomas (plasmablastic lymphoma, B).
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