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Review

Histopathology, pathogenesis and molecular genetics in primary central nervous system lymphomas

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Summary. Recent increases in the incidence of primary central nervous system lymphoma (PCNSL), a rare non-Hodgkin's lymphoma arising in the brain, have been noted in both immunodeficient and immunocompetent patients. Compared with lymphomas originating outside the central nervous system, the biology of PCNSL at the molecular or cytogenetic level has not been well characterized, yet it is important to thoroughly understand the etiology of this rare malignant lymphoma if effective therapies are to be developed. This review will focus on the epidemiology, clinical aspects, histopathology, pathogenesis, and molecular genetics of this aggressive, extranodal lymphoma in immunocompetent patients.

Key words: Primary central nervous system lymphomas, 6q LOH, R-PTP-κ, $p14^{ARF}$, $p16^{INK4A}$

Introduction

Primary central nervous system lymphoma (PCNSL) is defined as a diffuse lymphoma presenting in the brain or spinal cord in the absence of systemic lymphoma. Morphologically, 98% of these tumors are large B-cell, diffuse, lymphomas categorized as high-grade non-Hodgkin's type (Harris et al., 1994; Weber et al., 2000). Once considered a rare tumor, accounting for less than 1% of all systemic non-Hodgkin's lymphomas (NHLs), the incidence of PCNSLs in immunocompetent patients has been markedly increasing over the past decades (Cote et al., 1996). Although it remains relatively rare, it is, therefore, an increasingly important differential diagnosis for the physician presented with intracranial lesions.

Recent improvements in histopathology and immunohistochemical techniques have definitively established the lymphoid nature of both PCNSLs and systemic lymphomas. Because there are no lymph nodes or lymphatics within the nervous system, however, the pathogenesis and histogenetic origin of PCNSL in immunocompetent patients is still poorly understood regardless of the phenotypic similarities between CNS and non-CNS lymphomas.

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Epidemiology

The incidence of PCNSL has been increasing steadily since the 1970s. Data from the Surveillance, Epidemiology and End Results (SEER) database of the National Cancer Institute show that PCNSL increased more than 10-fold from 0.025/100000 in 1973 to 0.3/100,000 in 1991 and the forecasted incidence for the year 2000 is 0.5/100,000 (Corn et al., 1997). The PCNSL/glioblastoma rate was 1/250 in 1974 and rose to 1/36 in 1980, reaching 1/6 in 1991 (Eby et al., 1988). PCNSLs thus account for more than 6% of all intracranial tumors (Cote et al., 1996) and for 1-2% of all extranodal lymphomas (Fine et al., 1993; Miller et al., 1994).

The rising incidence of PCNSL can be explained, in part, by the increasing spread of the human immunodeficiency virus (HIV) over the last two decades and the more prevalent use of immunosuppressive agents. In fact, PCNSL is diagnosed in ~9.0% of the HIV-infected population (Rosenblum et al., 1988). The reason for the increased frequency of the disease in immunocompetent individuals, however, is less clear and cannot be explained by advances in neuroimaging or other diagnostic techniques. Neither have suspected environmental or behavioral risk factors been proposed. As PCNSL primarily affects individuals aged 60 and over, the general aging of the population might offer an explanation; however, the data indicate an increase across all age groups. For whatever reason or reasons, it appears that the number of patients with PCNSL is likely to continue to increase over the next decade.

Clinical aspects

To understand the specific issues related to the treatment and diagnosis of PCNSL, one must first

recognize the unique pathophysiology of the tumor. As previously mentioned, PCNSL has been described in patients at all ages, but most cases arise in the sixth decade with a male to female ratio of 1.5 (Fine et al., 1993). Because of the high replicative index, the interval between the onset of symptoms and diagnosis is usually short (1-3 months); focal motor and/or sensitive neurological deficits are frequent, with manifestations of intracranial hypertension, personality disturbances, and behavioral changes when the frontal lobe is affected. Systemic symptoms are very rare and epileptic seizures are less frequent than in glial tumors due to the preferential involvement of the white matter and deep structures as opposed to the cortex (Fine et al., 1993; Herrlinger et al., 1999).

In up to 70% of cases, the lymphomatous lesion is usually supratentorial and single at diagnosis, but frequently becomes multifocal in the late phase of the disease. The most frequent sites of origin are, in most cases, the basal ganglia, the corpus callosus, and/or the periventricular subependymal tissues. This peculiarity accounts for the frequent liquoral spread of neoplastic lymphocytes, even though cytological examination of the cerebrospinal fluid (CSF) is positive in no more than 40% of patients. Only 7% of PCNSL have an exclusively leptomeningeal localization (Lachance et al., 1991); a single and circumscribed involvement of the spine is a very sporadic finding (McDonald et al., 1995).

Histopathology

Histologically, PCNSL is indistinguishable from systemic NHL; furthermore, analysis of cell surface markers, including NCAM and integrins, is also identical to that of systemic lymphoma. Biologically, PCNSL behaves in an aggressive fashion, and it should be considered a high-grade tumor. Microscopically, PCNSL diffusely infiltrates brain parenchyma with an angiocentric growth pattern forming collars or perivascular cuffing by tumor cells; some tumors may even invade the blood vessel wall (Fig. 1A,B). In addition to malignant lymphoma cells, there are varying numbers of small, benign, reactive T-lymphocytes infiltrating the tumor; reactive astrocytes are also common. Tumor cells freely invade the normal surrounding brain, giving the appearance of encephalitis, and autopsy studies demonstrate widespread infiltration of normal tissue. Virtually all PCNSLs show a diffuse growth pattern; a follicular growth pattern has not been described.

The malignant cells are clearly identified as white blood cells with leukocyte common antigen (LCA) immunostaining, but a small number of PCNSLs may be immunonegative. These reactive infiltrative cells are typically CD4+ T-lymphocytes. According to the Revised European-American Classification of Lymphoid Neoplasm (REAL) classification (Harris et al., 1994), as modified in the WHO classification, PCNSLs belong to two main classes: those comprised of diffuse large cells, and those that contain high-grade Burkitt-like cells (Bataille et al., 2000). Approximately 98% of PCNSLs are CD20+ B-cell lymphomas (Fig. 1C), usually of the diffuse large-cell, immunoblastic, or lymphoblastic subtypes, and can be identified immunohistochemically with the B-cell marker L26. In a series of 226 patients, Blay and colleagues (Blay et al., 1998) found that more than 90% of PCNSLs fell within the Working Formulation groups designated G (diffuse large cells) and H (immunoblastic) with only 5% characterized as group J (Burkitt-type cells); only a very few were phenotypically low-grade small lymphocytic tumors. The majority of the tumors express monoclonal surface or cytoplasmic immunoglobulins, the most frequent combination being IgM/kappa.

In contrast, T-cell-rich B-lymphoma, true T-phenotype lymphoma (Villegas et al., 1997), CD30/Ki-1+ anaplastic large cell lymphoma, and primary Hodgkin's disease of the brain (Klein et al., 1999) are very rare and must be distinguished from true T-cell lymphoma. T-cell lymphomas only constitute about 2% of all PCNSL and are seen mainly in immunocompetent patients. They occur more frequently in posterior fossa locales, particularly in the cerebellum, and also show a propensity to arise in the leptomeninges. Some subsets of Ki-1 lymphomas are considered to be of T-cell origin.

The diagnosis of Hodgkin's disease rests on the identification of Reed-Sternberg cells confirmed by immunohistochemistry. Prior to more modern immunohistochemical techniques, many cases diagnosed as "primary intraparenchymal Hodgkin's disease", based on the presence of atypical immunoblasts resembling Reed-Sternberg cells, may have actually been large-cell anaplastic or pleomorphic lymphomas.

Angiotropic lymphomas, also known as intravascular lymphomas, affect multiple organ systems. (Fig. 1D) Accumulations of large B-cells within small and medium vessels lead to vascular occlusion and disseminated small infarcts. Absent or reduced expression of beta-2 integrins on tumor cells may contribute to an impaired capability for extravasation.

Pathogenesis

In immunosuppressed patients, PCNSL virtually always arises from Epstein-Barr virus (EBV)-infected B-lymphocytes (Forsyth et al., 1996; Lutz et al., 1994). The cells are latently infected after primary viral infection and reside within the individual for the remainder of their life. When either iatrogenic or acquired immunosuppression develops, loss of suppressor T-cells controlling the immortalized latently-infected lymphocytes allows these precursors to proliferate uncontrollably and develop into a neoplasm.

The pathogenesis in immunocompetent patients, however, is not so well-documented. The central nervous system (CNS) is usually considered an "immunological sanctuary" because it lacks lymphoid tissue. While T-lymphocytes normally traffic in and out of the CNS,

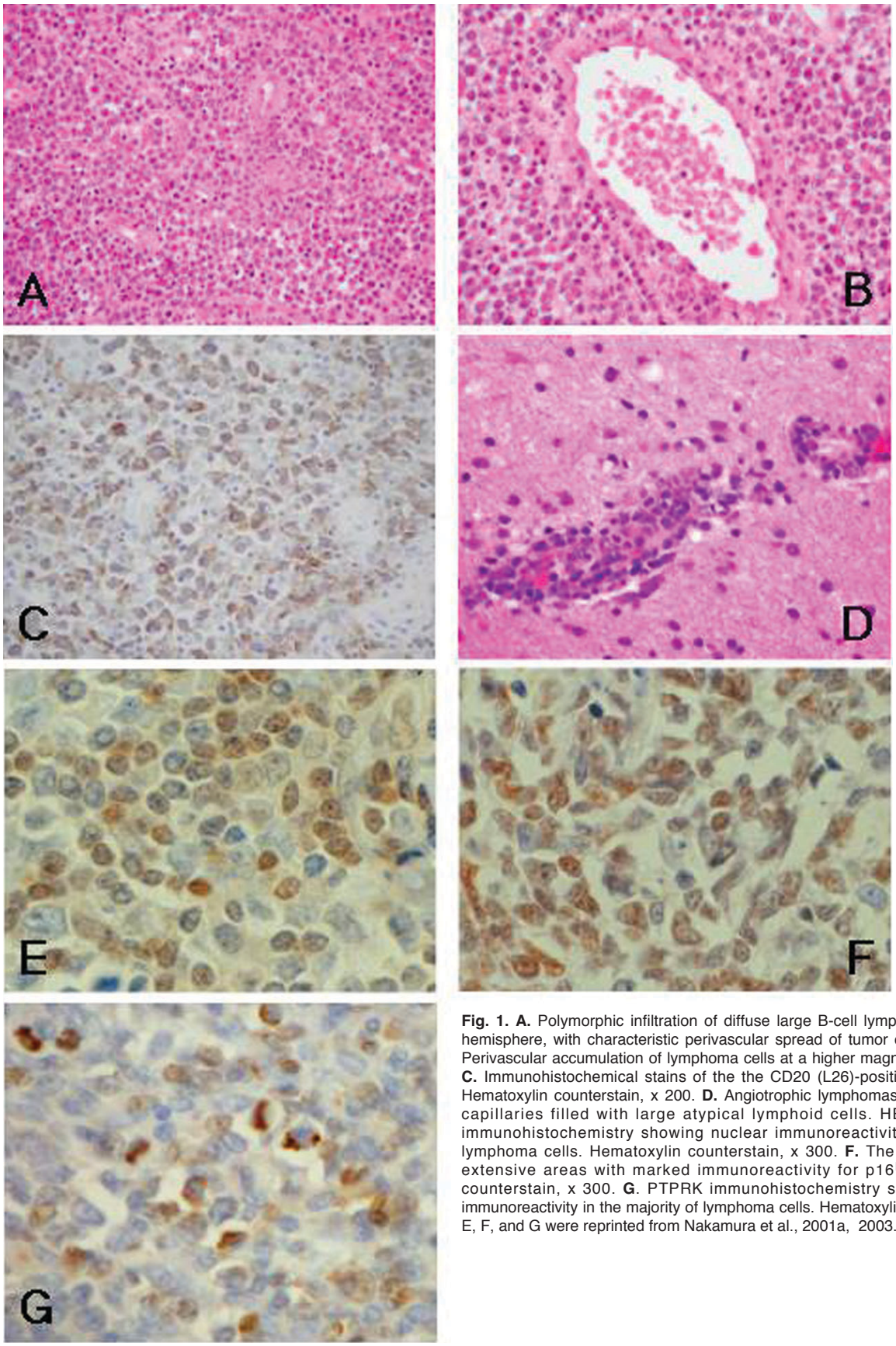

Fig. 1. A. Polymorphic infiltration of diffuse large B-cell lymphoma in the cerebral hemisphere, with characteristic perivascular spread of tumor cells. H-E, x 100. **B.** Perivascular accumulation of lymphoma cells at a higher magnification. H-E, x 200. **C.** Immunohistochemical stains of the the CD20 (L26)-positive B-cell phenotype. Hematoxylin counterstain, x 200. **D.** Angiotrophic lymphomas showing small brain capillaries filled with large atypical lymphoid cells. HE x 200. **E.** $p14^{ARF}$ immunohistochemistry showing nuclear immunoreactivity in the majority of lymphoma cells. Hematoxylin counterstain, x 300. **F.** The same case exhibits extensive areas with marked immunoreactivity for $p16^{INK4a}$. Hematoxylin counterstain, x 300. **G**. PTPRK immunohistochemistry showing cytoplasmic immunoreactivity in the majority of lymphoma cells. Hematoxylin counterstain, x 300. E, F, and G were reprinted from Nakamura et al., 2001a, 2003.

there is no normal traffic of B-lymphocytes. A known etiology or associated risk factor(s) for the development of PCNSL in immunocompetent patients has yet to be established, although several different hypotheses have been proposed:

1) PCNSL may arise from a systemic lymphoma that seeds multiple organs, including the brain. The immune system has the capacity to find and eradicate the systemic tumor, but the brain, an immunologically privileged site, gives sanctuary to the malignant lymphocytes within the CNS, thereby allowing tumor development. However, this seems unlikely, as there is no evidence of concomitant lymphoma in other immunologically privileged sites, such as the testes.

2) Trauma or infectious processes may attract peripheral blood lymphoid cells that are stimulated to proliferate locally and/or to undergo clonal selection and thus progress to a monoclonal neoplastic state. This is a plausible scenario; at present, however, we do not know which exogenous or endogenous antigenic stimulus might trigger what appears to be, at least in its early phase, an antigen-dependent clonal proliferation. Furthermore, inflammatory diseases almost exclusively attract T-lymphocytes while PCNSL is usually of B-cell origin, nor is the incidence of PCNSL increased in patients with inflammatory CNS diseases.

3) Lymphomatous cells generated in other tissues might occasionally develop adhesion molecules and acquire a preferential "homing" for cerebral endothelium. However, a comparison of adhesion molecules and other surface antigens expressed by both PCNSL and systemic NHL showed no significant differences (Jellinger et al., 1995; Paulus et al., 1993).

No data exist to support or refute any of these potential mechanisms.

Chromosomal abnormalities

The peculiar clinicopathological setting of PCNSL suggests the presence of molecular events distinct from non-CNS lymphomas which underlie the pathogenesis. In contrast to nodal and extranodal diffuse large B-cell lymphoma (DLBCL) originating outside the CNS, the cytogenetic profile of PCNSL has not been well characterized and seems inconsistent. Conventional chromosome banding analysis of PCNSL is hampered by the unavailability or scarcity of viable tumor cells obtained from stereotactically-derived minute tissue fragments during the diagnostic process. So far, karyotypes of only very few cases have been reported and these have not revealed any consistent findings (Itoyama et al., 1994; Zattara-Cannoni et al., 1998). Itoyama et al. (1994) showed that cytogenetic analyses of PCNSL cases revealed abnormalities on chromosomes 1, 6, 7 and 14, as well as translocations at 1;14, 6;14, 13;18, and 14:21, findings similar to those observed in nodal B-cell lymphoma.

The comparative genomic hybridization (CGH) technique has provided additional information on chromosomal gains and losses in PCNSL (Rickert et al., 1999; Weber et al., 2000; Harada et al., 2001). Gains in DNA copy number occurred in ~40% each of chromosomes 1q, 12, 18q, and on 23% of 7q, while losses of copy number were detected in 50% of chromosome 6q, 18% of 6p, and 15% of both 17p and 18p. Gains on 1q have also been found to be present in nodular DLBCL (Monni et al., 1996) and in DLBCL of the gastrointestinal tract (Barth et al., 1998) as well as in marginal zone B-cell lymphomas (Dierlamm et al., 1997) and follicular lymphomas (Tilly et al., 1994) by CGH. A candidate gene on 1q23-31, a frequently highly amplified region, has not been put forward; this region does correspond, however, to the location of the TRK/TRKC proto-oncogene. The most frequent alteration detected by CGH was gain of chromosome 12 with a commonly altered minimal overlapping region at 12p12–14. This locus harbors the MDM2/CDK4/GLI genes Interestingly, amplification of MDM2 is associated with DLBCL (Rao et al., 1998). Gain of chromosome 18 with a commonly altered overlapping region at 18q21–qter has also been frequently detected. The BCL2 gene is involved in this locus and is associated with a reciprocal translocation with the immunoglobulin heavy chain gene at 14q32 present in a third of DLBCL (Houldsworth et al., 1996; Monni et al., 1997; Rao et al., 1998). The next most frequent gain is of chromosome 7q, the amplification of which has also been found in a number of B-cell neoplasms, in general (Werner et al., 1997), as well as in extranodal systemic DLBCL (Houldsworth et al., 1996; Monni et al., 1996), in follicular lymphomas (Avet-Loiseau et al., 1997), in high-grade MALT (Chan et al., 1998) and in mediastinal thymic B-cell lymphomas (Joos et al., 1996). Here, however, candidate proto-oncogenes and tumor suppressor genes have not yet been located.

Allelic losses on the long arm of chromosome 6, i.e., 6q, occur in 20-40% of both systemic malignant non-Hodgkin's lymphomas and acute lymphoblastic leukemias, and are among the most frequent chromosome aberrations found in these diseases. Two regions within 6q, specifically 6q21-23 and 6q25-27, have been isolated which may contain different tumor suppressor genes (TSGs) involved in lymphoma development (Offit et al., 1993; Hauptschein et al., 1998; Merup et al., 1998). Interestingly, the presence of 6q deletions correlates with poor patient prognosis in systemic lymphomas, suggesting that the identification of the putative target TSGs on 6q may provide particular markers of clinical significance (Tilly et al., 1994; Whang-Peng et al., 1995). Using CGH methods on analysis of large B-cell-type CNS lymphomas, Weber et al. (2000) further reported that 6q showed losses of genomic material in fully 47% of informative cases, with a commonly deleted region mapping to 6q21-22. When these earlier studies were performed, however, fine deletion mapping on 6q by LOH analysis had not yet been done in PCNSL. Recently, we subjected samples of PCNSLs to fine deletion mapping of 6q in order to

identify any minimally conserved regions of deletions (MCRDs) that might be present and detected a minimum deletion interval (~140kb) on chromosome band 6q22-23 likely to contain a lymphoma-related TSG. We also determined that deletions within this region occurred in 66% of samples analyzed, making it a more frequent phenomenon than that found at other locations in lymphomas (Nakamura et al., 2003). LOH in 6q in PCNSLs was also analyzed at the time of relapse in 4 patients (Nakamura et al., 2003); in all 4 patients, the 6q structure was the same as in their first presentation, suggesting that, albeit in a limited sample size, 6q deletions may be an initial event in PCNSL pathogenesis that occurs less frequently during progression.

Molecular genetics

Information on the molecular pathogenesis of primary CNS DLBCL arising in immunocompetent patients is also very limited, in contrast to non-CNS DLBCL or AIDS-related CNS DLBCL. We had previously found the most frequent abnormality in our series of PCNSL to be either homozygous deletion of or promoter hypermethylation of the $p14^{ARF}$ (56%) and $p16^{INK4a}$ (61%) genes (Nakamura et al., 2001a) and, further, that these alterations correlated with gene expression (Fig. 1E,F). We detected $p14^{ARF}$ homozygous deletion in 50% of samples, and, with a single exception, the $p14^{ARF}$ promoter was unmethylated independent of methylation in $p16^{INK4a}$. Within this context, it is of interest that Baur et al. (1999) found frequent methylation-induced silencing of $p15^{INK4b}$ and $p16^{INK4a}$ in non-CNS B-cell and T-cell lymphomas, while methylation silencing of $p14^{ARF}$ appeared to be extremely rare. Meléndez et al. (2000) also pointed out that expression of $p19^{ARF}$, which is the murine homolog of $p14^{ARF}$, was lost or reduced in a significant percentage of murine primary lymphomas, whereas CpG island methylation in $p19^{ARF}$ was an infrequent event. Thus, in PCNSL $p14^{ARF}$ homozygous deletion is more likely to be essential for $p14^{ARF}$ inactivation than is promoter hypermethylation. The $p16^{INK4a}$ and $p14^{ARF}$ genes are frequently co-deleted in human neoplasms and this was also the case in our series of PCNSLs. However, cases with $p14^{ARF}$ deletion alone were also encountered and at a higher rate than $p16^{INK4a}$ deletions reported for other human neoplasms (Xing et al., 1999; Nakamura et al., 2001b). This conclusion is supported by studies of mice lacking $p19^{ARF}$ expression alone through selective disruption of exon 1ß; these mice develop tumors at several sites, including lymphomas, sarcomas, and gliomas (Kamijo et al., 1999).

$p14^{ARF}$ plays a major role in the p53 pathway by binding specifically to MDM2, resulting in stabilization of both p53 and MDM2 (Pomerantz et al., 1998; Stott et al., 1998). With regard to MDM2 expression, PCNSL appear similar to systemic lymphomas, showing only occasional gene amplification (Quesnel et al., 1994; Cobbers et al., 1998). Growth arrest induced by $p14^{ARF}$ is, therefore, p53-dependent. Recent studies on the INK4a/ARF locus as a regulatory region for both the $p16^{INK4a}$/RB1 and $p14^{ARF}$/p53 pathways indicate that p53 mutations may be more rare in tumors with inactivation of this locus than in those with wild-type INK4a/ARF genes (Pomerantz et al., 1998). In our series of PCNSLs, $p14^{ARF}$ alterations and p53 mutations appeared to be unrelated and an inverse correlation between $p14^{ARF}$ and p53 expression is not always detected as it is, for example, in leukemia-lymphoma cell lines and in large B-cell lymphomas (Moller et al., 1999). Although inactivation of the p53 gene is a relatively common phenomenon in lymphomas outside the central nervous system, being found in 20-40% of such neoplasms (Hernandez et al., 1996), p53 mutations appear to be extremely rare in PCNSL (Cobbers et al., 1998; Zhang et al., 1998; Nakamura et al., 2001a). It has been suggested that the molecular pattern of these tumors may depend on the order of events (Stott et al., 1998); when $p14^{ARF}$ alterations occur early in the development of PCNSL, the tumors may retain wild-type p53.

More than 50% of high-grade systemic NHLs lack retinoblastoma protein (pRB) expression (Weide et al., 1994). In contrast, Cobbers et al. (1998) reported that PCNSL showed strong nuclear immunoreactivity in all of their 20 samples. Loss of pRB expression was found in 22% of the cases we examined. In both glioblastomas (Nakamura et al., 2001c) and pituitary adenomas (Simpson et al., 2000), a clear correlation was detected between RB1 homozygous deletion and/or promoter hypermethylation and loss of immunohistochemical pRB expression. Our study suggests that this might also be the case for PCNSL, with promoter methylation being the underlying cause for loss of RB1 expression (Nakamura et al., 2001a).

$p21^{Waf1}$ alterations in human lymphomas have been analyzed in only a few studies. A role for $p21^{Waf1}$ deletions and loss of expression in aggressive non-CNS lymphomas was proposed by Pinyol et al. (1997), but others failed to identify mutations of this gene in a large series of lymphoid neoplasms (Gong et al., 1995). $p21^{Waf1}$ expression in PCNSL was detected in only ~50% of lesions (Cobbers et al., 1998; Nakamura et al., 2001a). However, even in those samples lacking $p21^{Waf1}$ expression promoter hypermethylation of $p21^{Waf1}$ was not detected and $p21^{Waf1}$ point mutations seem to be very rare in human tumors (Shiohara et al., 1994). It could be that p21Waf1 expression is regulated at the transcriptional level, although the significance of $p21^{Waf1}$ in the development of lymphomas is still unclear.

$p27^{Kip1}$ is commonly analyzed in human cancers due to its function as an important cyclin-dependent kinase inhibitor impacting on cell passage through the G1 as well as G2 restriction points in the cell cycle. Specific alterations in the $p27^{Kip1}$ gene, including mutations and homozygous deletions, are exceedingly rare in human cancers, including systemic NHLs

(Morosetti et al., 1995). In a series of 18 PCNSL cases, we found 4 to be immunonegative for $p27^{Kip1}$ expression; in 2 of those cases, $p27^{Kip1}$ hypermethylation was also detected. However, aberrant $p27^{Kip1}$ methylation is not the only mechanism that can cause reduced levels of $p27^{Kip1}$ expression, and epigenetic influences could also play a role in pathogenesis.

Yang et al. (1996) showed that TGF-ß1 inhibits human keratinocyte proliferation in vitro, possibly through induction of PTPRK gene expression and further suggested that PTPRK might be involved in the formation and maintenance of intact adherens junctions via dephosphorylating ß-catenin and γ-catenin/plakoglobin or cadherins. PTPRK has also been indicated to be involved cell proliferation, tumor invasiveness, and metastatic spread (Fuchs et al., 1996). We recently demonstrated frequent allelic losses on chromosome 6q22-23 in PCNSLs, and further demonstrated non-expression of PTPRK correlating with 6q22-23 deletion, implicating PTPRK as a strong candidate for a major TSG in PCNSL (Nakamura et al., 2003). LOH at 6q22-23 and loss of PTPRK protein expression was observed in 76% of PCNSL specimens examined (Fig. 1G)

Southern blot analyses failed to detect alterations of several oncogenes in PCNSL (Larocca et al., 1998; Nozaki et al., 1998; Zattara-Cannoni et al., 1998). Specific oncogenes appear to be targeted by amplifications or breakpoints in systemic DLBCL (Chaganti et al., 2000; King et al., 2000), including REL particularly in the chromosomal region 2p13-15, BCL6 in 3q27, c-Myc in 8q24, or BCL2 in 18q21, while genetic alterations in such genes as $p21^{Waf1}$, MDM2, CDK4, and CCND1 have not been detected (Cobbers et al., 1998; Nakamura et al., 2001a).

Prognosis

A patient age of less than 60 years and good performance status are the most important favorable prognostic factors in multivariate analysis of PCNSL, while worse outcomes are predicted by involvement of the brain stem or spine, lesion multiplicity, and increased CSF protein levels (Pollack et al., 1989; DeAngelis et al., 1992; Fine et al., 1993; Abrey et al., 1998; Bataille et al., 2000). Ocular extension and high levels of LDH and ß2-microglobulin in the CSF do not appear to be significant. Contrary to other extranodal NHLs, different histological subtypes do not correlate with differences in clinical course and response to therapy, therefore no treatment modification is required (Jellinger et al., 1995).

To our knowledge, systematic evaluation of the prognostic significance of oncogene or TSG alterations in primary CNS DLBCL is lacking in the literature, although this evaluation is critically needed. We have reported aberrations of several tumor-related genes (Nakamura et al., 2001a), but have failed to find significant correlation between clinical course and $p14^{ARF}$, $p16^{INK4a}$, RB1, and $p27^{Kip1}$ alterations, in particular. Several other investigators suggest that deletion of 6q may be correlated with a shorter survival time, at least in non-CNS lymphomas (Tilly et al., 1994; Whang-Peng et al., 1995), and our relapse data does not contradict the impression that 6q deletions have a stronger influence on the clinical behavior of PCNSL than on that of systemic lymphomas (Nakamura et al., 2003). In addition, we noticed a tendency toward earlier death in patients with tumors having reduced PTPRK expression, implying a potential prognostic value in tissue PTPRK status and supporting the possible tumor suppressor function of the gene (Fig. 2).

Conclusions

As we learn more about the molecular biology of PCNSLs, it becomes obvious that there are likely to be many subtypes of tumors with characteristic behaviors and subsequent patient prognosis. What also becomes apparent is that defining molecular pathogeneses will

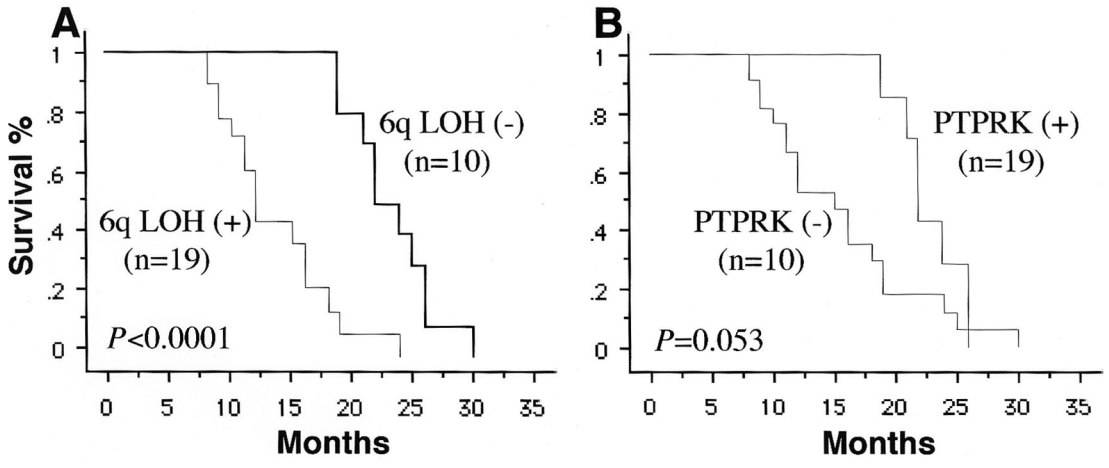

Fig. 2. Kaplan-Meier curves for overall survival in relation to LOH on 6q **(A)** or PTPRK expression **(B)** of 29 patients with PCNSL. (Reprinted from Nakamura et al., 2003).

require years of research into the genetic histogenesis of these tumors and the consistent and detailed correlation of different genetic subtypes with individual patient outcomes.

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