NEOPLASIA
www.neoplasia.com

Volume 20 Number 10 October 2018 pp. 1059–1069 1059

# Analysis of Genomic Alteration in Primary Central Nervous System Lymphoma and the Expression of Some Related Genes

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## Abstract

Primary central nervous system lymphoma (PCNSL) is a rare and special type of non-Hodgkin lymphoma. The treatment of PCNSL is comprehensive, combining surgery, radiotherapy, and chemotherapy. However, the outcome is poor because of its high invasiveness and rate of recurrence. We analyzed 22 cases of PCNSL using next-generation sequencing (NGS) to detect 64 candidate genes. We used immunohistochemical methods to analyze gene expression in 57 PCNSL samples. NGS showed that recurrent mutations in *KMT2D* and *CD79B*, components of the NF-κB pathway, accounted for 65% of total mutations in PCNSL samples. The most frequent mutated gene was *PIM1* (77.27%, 17/22), followed by *MYD88* (63.64%, 14/22), *CD79B* (69.09%, 13/22), and *KMT2D* (50.00%, 11/22). Mutations of the *CD79B* gene were associated with an inferior progression-free survival (PFS), and *GNA13* gene mutations were associated with a shorter PFS and overall survival (OS) in PCNSL patients ($P < .05$). *PIM1* and *MYD88* were highly expressed in PCNSL patients and were related to their OS time. *MYD88* overexpression might be an independent and poor prognostic predictor of OS time. In summary, we identified highly recurrent genetic lesions in *CD79B* and *KMT2D*, components of the NF-κB pathway, in PCNSL and validated the expression of *PIM1* and *MYD88* related to poor survival, thereby providing novel insights into the pathogenesis and precision medicine of PCNSL.

*Neoplasia (2018) 20, 1059–1069*

## Introduction

Primary central nervous system lymphoma (PCNSL) is derived from the central nervous system (CNS) and can include the brain parenchyma, spinal cord, eyeball, cranial nerve, and meninges but does not include dural lymphomas (such as follicular cell lymphoma and mantle cell lymphoma), intravascular B-cell lymphoma, immunodeficiency, or secondary to CNS lymphoma. It is a special type of non-Hodgkin lymphoma (NHL), accounting for 4%-6% of extracellular lymphomas and 1% of adult NHLs. More than 95% of PCNSL cases are diffuse large B-cell lymphoma (DLBCL), and other rare types include T-cell lymphoma and Burkitt lymphoma [1–3] The incidence of PCNSL increases with age, and it is becoming more common in aging populations, with a median age of 55-65 years [4]. The etiology of PCNSL is unclear, but it was reported to be related to EB or HIV infection, organ transplantation, or other diseases leading

Abbreviation: ABC, activated-B Cell; BTK, Bruton's tyrosine kinase; CNS, Central nervous system; CLL, chronic lymphocytic leukemia; DLBCL, Diffuse Large B-cell lymphoma; DAB, diaminobenzidin; DNMT, DNA methyltransferase; GO, gene ontology; GCB, germinal center cell like; HR, hazard ratio; HDAC, histone deacetylase; IHC, Immunohistochemistry; LDH, lactate dehydrogenase; MYD88, Myeloid Differentiation Factor 88; MCL, mantle cell lymphoma; NHL, Non-Hodgkin Lymphoma; NGS, next generation sequencing; OS, overall survival; ORR, overall response; PFS, progression-free survival; PIM1, proviral integration of moloney murine leukemia virus; PCNSL, Primary Central Nervous System Lymphoma; PKC, Protein kinase C; SNPs, single nucleotide polymorphisms

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Received 22 May 2018; Revised 31 August 2018; Accepted 31 August 2018



1476-5586
https://doi.org/10.1016/j.neo.2018.08.012

to immunodeficiency [5]. PCNSL exists behind the blood-brain barrier and blood-cerebrospinal fluid barrier; therefore, traditional and single-treatment methods do not effectively control tumor development. Current treatment methods include high-dose methotrexate-based chemotherapy, radiotherapy, targeted therapy, and stem cell transplantation [3,6,7]. However, for most PCNSL patients, the overall prognosis is poor, with a median overall survival time of 1-4 years, and the prognosis is significantly worse than for other NHLs outside the brain [8,9].

Because of the rarity of PCNSL and the limited availability of biopsy tissues, the pathogenesis of PCNSL is still poorly understood, especially in the genomics research field in China. This has hindered the development and treatment of PCNSL. Advances in next-generation sequencing (NGS) technology have enabled the effective and comprehensive analysis of the molecular composition and function of various solid tumors and hematological malignancies, including PCNSL. Studies have shown that in PCNSL, *MYD88* and *CD79B* are the most frequently mutated genes (30%-83%), and approximately 16% of the mutations are targeted to the *CARD11* coiled helix domain leading to the inactivation of *TFNAIP3* (3%) [10–12]. Transcriptome studies reported that disordered genes in PCNSL are involved in the IL-4/JAK/STAT6, cell adhesion-related, unfolding protein response, and apoptosis-related signaling pathways [13]. Replication of copy number variation studies found that PCNSL patients have frequent chromosome deletions, especially in chromosome regions 6q, 6p21.32, and 9p21 [14]. However, which mutant genes are present in PCNSL are still unclear.

The NF-κB signaling pathway belongs to the receptor protein hydrolase-dependent receptor signaling pathway. In recent years, many studies have shown that the NF-κB pathway has a significant role in tumor occurrence, development, proliferation, differentiation, apoptosis, invasion, and metastasis [15]. In DLBCL, NF-κB is a common downstream effector molecule of many signaling pathways, including the BCR, TLRs, NOTCH, and JAK-STAT signaling pathways, all of which can activate their downstream factor NF-κB. Furthermore, interactions between different pathways form a complex signaling network and jointly promote the proliferation and differentiation, apoptosis, angiogenesis, invasion, metastasis, resistance, and other pathophysiological processes of lymphoma cells [16–18]. Other studies reported high-frequency mutations and abnormal activation of the NF-κB pathway in lymphoma patients [19,20], providing new insights to the pathogenesis and prognosis of lymphoma.

In this study, we investigated the genomic alterations and related gene expressions in PCNSL patients to provide new insights into the mechanisms of lymphomagenesis and potential prognostic factors or treatment opportunities for PCNSL.

## Materials and Methods

### *Patients*

A cohort of 57 specimens was obtained from Xiangya Hospital of Central South University from June 2012 to September 2016. Twenty-three of the latest 2-year diagnosis samples were used for DNA extraction and NGS. All cases of PCNSL were confined within the CNS (stage IE), were human immunodeficiency virus unrelated, and fulfilled the World Health Organization criteria for diagnosis. None of the patients had evidence of immunodeficiency. We also collected 20 cases diagnosed with lymphadenitis, which were used as the control group. All paraffin-embedded tumor specimens were collected in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national).

The clinical information included gender, age, PCNSL International Prognostic Index (IELSG), lactate dehydrogenase (LDH) level, type, treatment regimens, and survival time. All patients had complete clinical and follow-up data from the day of diagnosis to June 2017. Treatment response was evaluated using imaging techniques. The progression-free survival (PFS) and overall survival (OS) of these patients were calculated.

### *DNA Isolation*

Twenty-three formalin-fixed paraffin-embedded (FFPE) tissue samples were obtained from archived material. FFPE material contained at least 70% tumor cells. DNA was extracted from a certain amount of wax roll samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA concentrations were measured with Qubit Fluorometer 2.0 (Life Technologies, Darmstadt, Germany). Sufficient amounts of DNA for further analysis were isolated from all archived FFPE samples. One patient was excluded from further analysis because of poor DNA quality.

### *Next-Generation Sequencing*

The sequencing platform was provided by Burning Rock Biomedical Company (Guangzhou, China) with a panel of 64 lymphoma-related genes (Supplementary Table 1), which were related to lymphoma pathogenesis and targeted therapy. We used the probe hybridization enrichment method to detect the exon regions of all the genes and the intron region of parts of the genes. According to the manufacturer's protocol, the library preparation was performed using approximately 200 ng of genomic DNA for sequencing on the Illumina MiSeq system (Illumina Inc., San Diego, CA). Library size and quality were demonstrated with the Agilent High sensitivity DNA Kit (Agilent, Santa Clara, CA).

### *Immunohistochemistry*

The streptavidin-peroxidase–conjugated method was used for the detection of PIM1 and MYD88 expression. Resected tissue specimens of 4-μm thickness were deparaffinized in xylene and rehydrated through a gradient of alcohol and deionized water. Heat antigen retrieval was performed using ethylenediaminetetraacetic acid (pH 9.0) for 20 minutes followed by 3% hydrogen peroxide for 20 minutes and serum blocking for 30 minutes. Tissues were incubated overnight at 4°C with anti-PIM1 rabbit polyclonal antibody (Abcam, ab75776, USA, dilution 1:100) and anti-MYD88 rabbit monoclonal antibody (Abcam, ab33739, USA, dilution 1:500). Then, it was incubated with a biotin-conjugated secondary antibody for 30 minutes at 37°C. Slides were incubated in DAB for 1-2 minutes, and after 30 seconds of counterstaining with hematoxylin, the slides were dehydrated and mounted.

PIM1 and MYD88 immunohistochemistry expression was scored using a semiquantitative system based on the intensity and percentage of staining [21,22]: negative, weak, moderate, or strong intensity. Negative and weak intensities were regarded as low expression; moderate and strong intensities were considered as high expression. We randomly selected five high-magnification (400×) areas of high-quality staining for each slide. Immunostaining results were independently evaluated by two pathologists who were blinded to the clinicopathological features. Appropriate positive and negative controls were included in the IHC assay.