Cell of origin, genomic profiling and metabolic examination of the primary central nervous system lymphomas

PhD Theses

Dóra Marosvári M.D.

Semmelweis University
Doctoral School of Pathological Sciences

Supervisors:
- Lilla Reiniger, M.D, Ph.D.
- Csaba Bödör, Ph.D.

Official reviewers:
- Lajos Gergely M.D, Ph.D.
- Ágnes F. Semsei, Ph.D.

Head of the Final Examination Committee:
- Edit Buzás, M.D, corr. member of the HAS

Members of the Final Examination Committee:
- Gábor Mikala, M.D, Ph.D.
- Dániel Erdélyi, M.D, Ph.D.

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I. Introduction

Primary central nervous system lymphoma (PCNSL) is a rare, aggressive form of extranodal non-Hodgkin lymphoma; arising exclusively within the brain, spinal cord, leptomeninges and eye. Histologically, the majority of PCNSLs is characterized as diffuse large B-cell lymphoma (DLBCL). In contrast, secondary central nervous system lymphoma (SCNSL) is defined as secondary central nervous system involvement in patients with systemic lymphoma.

It is well-known, that sub-classification of systemic DLBCLs into cell of origin (COO) categories (activated B-cell like- ABC; germinal center B-cell like-GCB) has prognostic and potential therapeutic implications. Many novel agents, e.g. lenalidomide, bortezomib and ibrutinib, show more pronounced activity in ABC DLBCL, while BCL2, BCL6 and EZH2 inhibitors seem to be more effective in GCB DLBCL. The gold standard method for GCB/ABC classification of DLBCL is based on gene expression profiling (GEP), however various immunohistochemistry (IHC) based

algorithms have been described in the past few years. Unfortunately, the GEP method is impractical for routine use and IHC algorithms seem to have low reproducibility. The NanoString Lymphoma Subtyping Test (LST) assay was developed to establish a formalin fixed paraffin embedded (FFPE) tissue compatible, gene expression based test for the molecular subtyping of B-cell lymphomas.

The molecular subtype of PCNSL has been studied by different methodological approaches with conflicting conclusions. Based on several IHC studies, an ABC-like immunophenotype is typical, while gene expression profiling studies indicate that PCNSLs are distributed among the spectrum of systemic DLBCLs with roughly equal proportion of ABC and GCB cases.

Recent studies profiling the genomic background of PCNSL identified multiple recurrently mutated genes. These genes show considerable overlap with the mutational targets identified in systemic DLBCL. The most frequently mutated genes include members of the NF-κB pathway/B-cell receptor signaling (i.e. *MYD88*, *CD79B*, *CARD11*), as well as mutations of *PIM1*,

PRDM1 and *TBL1XR1* genes.

Dysregulation of the mTOR pathway, which is a key regulator of various cellular functions including proliferation, survival, and cell growth, has been recognized as a critical event in the development of different malignancies, including systemic DLBCL. Detection of phospho- ribosomal S6 (p-S6) is commonly used as a marker of mTOR pathway activity, however it is known that S6 protein may also be phosphorylated independently of mTOR via other kinases, such as PAS domain-containing serine/threonine-protein kinase (PASK). Nevertheless, there is a very limited data on mTOR pathway activity in PCNSL.

Recently, numerous new prognostics markers and potential therapeutic targets have been identified in PCNSL. These novel findings will most likely support and drive personalized therapeutic decisions during the management of PCNSL patients.

II. Aims

- To determine the cell of origin of primary and secondary central nervous system lymphoma by the NanoString LST assay.
- To compare the cell of origin results obtained by the NanoString LST assay and the standard immunohistochemistry algorithm in primary and secondary central nervous system lymphoma.
- To determine the mutational profile of *CARD11*, *CCND3*, *CD79B*, *C-MYC*, *CSMD2*, *CSMD3*, *IRF4*, *KMT2D*, *MYD88*, *PAX5*, *PIM1*, *PRDM1*, *PTPRD* and *TP53* genes by targeted ultra-deep next-generation sequencing in primary and secondary central nervous system lymphoma.
- To establish correlations among cell of origin, genomic profile and survival in primary and secondary central nervous system lymphoma.
- To determine the mTOR pathway activity in primary central nervous system lymphoma.
- To examine mTOR independent phosphorylation of the S6 ribosomal protein.

III. Materials and methods

III/1. Tissue samples

FFPE brain biopsy specimens from 81 patients with PCNSL and 18 patients with SCNSL were analyzed in this study. Tissue samples were obtained from three centers: (i) 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary; (ii) Department of Pathology, University of Pécs, Pécs, Hungary and (iii) Division of Neuropathology, The National Hospital for Neurology and Neurosurgery, University College London Hospitals, United Kingdom. Survival data was available in 65 PCNSL and 17 SCNSL cases.

III/2. Cell of origin

RNA isolation from 77 PCNSL and 17 SCNSL samples was performed, followed by determination of the molecular subtype using the NanoString LST assay. The method involves mixing RNA with reporter and capture

probes, followed by hybridization. After hybridization the complexes are bound and aligned in the nCounter Cartridge. Finally, nCounter Digital Analyzer collects the data by taking images of the fluorescent reporters and color codes are counted for each target molecule. For each RNA sample, a Linear Predictor Score (LPS) is calculated using a weighted sum of the gene expression. The LPS is compared against thresholds that define LPS value cut-offs for the assignment of ABC or GC subtype, or Unclassified category within an equivocal zone.

III/3. Mutational analysis

Genomic DNA was extracted from 64 PCNSL and 12 SCNSL samples. Mutation profiles of 14 genes (*CARD11*, *CCND3*, *CD79B*, *CSMD2*, *CSMD3*, *IRF4*, *KMT2D*, *C-MYC*, *MYD88*, *PAX5*, *PIM1*, *PRDM1*, *PTPRD* and *TP53)* were determined by targeted ultra-deep next-generation sequencing (NGS) using a HiSeq 4000 Instrument (Illumina). Following the bioinformatic analysis, a subset of somatic variants with variant allele frequency of >20% was validated by bidirectional Sanger

sequencing.

III/4. mTOR pathway activity

FFPE biopsy specimens from 31 cases of PCNSL and 51 cases of nodal DLBCL were included in our immunohistochemistry study. We measured mTOR activity with immunohistochemistry for p-mTOR and its downstream effectors (p-S6, p-4E-BP1, p(T389)-p70S6K1), and evaluated alternative S6 phosphorylation pathways with p-RSK, PASK and p(T229)-p70S6K1 antibodies. Finally, using a DLBCL cell line (BHD1), we examined the impact of PASK inhibitor (1μM, BioE-1115, Calbiochem) on p-S6 expression, measured by Western blot and flow cytometry.

IV. Results

IV/1. Molecular subtypes of brain lymphomas

Using the Hans algorithm, 95% of the PCNSL cases (73/77) showed non-GCB phenotype, with 5% of the patients (4/77) presenting with GCB phenotype. In contrast, the LST-assay identified only 80.5% (62/77) of the cases as ABC subtype, with 13% (10/77) GCB and 6.5% (5/77) UC subtypes, respectively.

As for the SCNSL cases, 47% (8/17) showed non-GCB and 53% (9/17) showed GCB phenotype with the Hans algorithm. The ratio was identical using the LST-assay, with 47% (8/17) ABC and 53% (9/17) GC subtypes. However, a single GCB and a single ABC case did not match when comparing the readouts of the Hans algorithm and the LST-assay.

IV/2. Mutation profiles of PCNSL and SCNSL

The most frequently mutated genes in the PCNSL cohort included *MYD88* (66%), *PIM1* (41%), *KMT2D*

(31%) and *PRDM1* (30%). *PRDM1* (50%), followed by *MYD88* (42%) and *PIM1* (25%) were the most frequently mutated target genes in the SCNSL cohort.

In PCNSL, *IRF4*, *CD79B*, *MYC*, *CARD11*, *CSMD2* and *CSMD3* mutations were observed exclusively in ABC cases. While, in PCNSL cases with GCB subtype, mutations of *TP53*, *PAX5* and *CCND3* appeared more frequent compared to the ABC cases.

The survival of the patients with PCNSL and SCNSL did not show a significant difference. Interestingly, the molecular subtypes did not have an impact on the survival of patients, with overall survival showing no differences between the GCB and ABC subtype neither in the entire cohort nor in the PCNSL cases. In contrast, cases with *CD79B* mutation showed significantly shorter overall survival compared to cases with a wild type *CD79B* gene, in all brain lymphomas and in PCNSL cases. Furthermore, *MYD88 and CCND3* mutations were associated with a tendency for longer overall survival in cases of PCNSL and in all brain lymphomas.

IV/3. mTOR activity in PCNSL

In this study, we demonstrated that the mTOR pathway is probably inactive in the majority of PCNSLs. While 83.9% of PCNSL cases showed p-S6 expression, mTOR pathway activity was observed only in 25.8% of the PCNSL cases. These results suggest that other proteins independent of mTOR pathway may phosphorylate S6, thus we measured the activity of three mTOR independent kinases (p-RSK, PASK and p(T229)-p70S6K1). Only PASK protein was found to be expressed by the tumor cells in all examined PCNSL cases. Inhibition of PASK using PASK inhibitor greatly reduced the level of p-S6 in BHD1 cell cultures as confirmed by Western blot and flow cytometry analysis.

V. Conclusions

- Molecular subtyping of primary central nervous system lymphoma revealed a higher proportion with GCB subtypes compared to previous data.
- We determined the mutational profile of primary and secondary central nervous system lymphoma. In PCNSL cases the most frequently mutated genes were *MYD88* (66%), *PIM1* (41%), a *KMT2D* (31%) and *PRDM1* (30%). While in SCNSL cases the most frequently mutated genes was the *PRDM1* (50%), followed by the mutations of *MYD88* (42%), *PIM1* (25%) and *KMT2D* (17%).
- In primary central nervous system lymphoma mutations of *IRF4*, *CD79B*, *C-MYC*, C*ARD11*, *CSMD2* and *CSMD3* were exclusively detected in ABC cases. In cases with GCB subtype, mutations of *TP53*, *PAX5* and *CCND3* appeared more frequent compared to the ABC cases.
- The cell of origin of brain lymphomas did not have an impact on the survival.

- Cases with *CD79B* mutation showed significantly shorter overall survival compared to cases with a wild type *CD79B* gene.
- *MYD88* and *CCND3* mutations were associated with a tendency for longer overall survival in cases of all brain lymphomas and PCNSL.
- The mTOR pathway activation is not typical in PCNSL, however mTOR independent pathways may be involved in S6 phosphorylation
- The PAS domain-containing serine/threonine-protein kinase (PASK) may contribute to S6 phosphorylation.

VI. List of publications

VI/1. Publications in connection with the dissertation:

1. **Marosvári D**; Nagy N; Kriston Cs; Deák B; Hajdu M; Bödör Cs; Csala I; Bagó AG; Szállási Z; Sebestyén A; Reininger L. Discrepancy Between Low Levels of mTOR Activity and High Levels of P-S6 in Primary Central Nervous System Lymphoma May Be Explained by PAS Domain-Containing Serine/Threonine-Protein Kinase-Mediated Phosphorylation. *Journal of neuropathology and experimental neurology*. 77: 268-273. (2018). **IF: 3.460**

2. Bödör Cs; Alpár D; **Marosvári D**; Galik B; Rajnai H; Bátai B, Nagy Á, Kajtár B, Burján A; Deák B; Schneider T; Alizadeh H; Matolcsy A; Sebastian B; Storhoff J; Chen N; Liu MD; Ghali N; Csala I; Bagó AG; Gyenesei A; Reiniger L. Molecular subtypes and genomic profile of primary central nervous system lymphoma. *Journal of Neuropathology and Experimental Neurology*. (2019) *doi: 10.1093/jnen/nlz125.* **IF: 3.460***

VI/2. Other publications

1. Gángó AP; Alpár D; Galik B; **Marosvári D**; Kiss R; Fésüs V; Aczél D; Eyüpoglu E; Nagy N; Nagy Á; Krizsán Sz; Reiniger L, Farkas P; Kozma A; Ádám E; Tasnády Sz; Réti M; Matolcsy A; Gyenesei A; Mátrai Z; Bödör Cs. Dissection of subclonal evolution by temporal mutation profiling in chronic lymphocytic leukemia patients treated with ibrutinib. *International journal of cancer.* 146: 85-93. (2019). **IF: 4.982***

2. Szurián K; Csala I; **Marosvári D**; Rajnai H; Dezső K; Bödör Cs; Piurkó V; Matolcsy A; Reiniger L. EZH2 is upregulated in the proliferation centers of CLL/SLL lymph nodes. *Experimental and molecular pathology*. 105: 161-165. (2018). **IF: 2.350**

3. Fesüs V; **Marosvári D**; Kajtár B; Király PA; Demeter J; Gurbity Pálfi T; Egyed M; Plander M; Farkas P; Mátrai Z; Matolcsy A; Bödör Cs. A TP53-mutáció-analízis jelentősége krónikus lymphocytás leukaemiában [TP53 mutation analysis in chronic lymphocytic

leukaemia]. Orvosi hetilap. 158: 220-228. (2017). **IF: 0.322**

4. Kiss R; Király PA; Gaál-Weisinger J; **Marosvári D**; Gángó AP; Demeter J; Bödör Cs. A krónikus mieloid leukémia molekuláris monitorozásának aktuális kérdései. [Molecular monitoring of myeloid leukemia]. Magyar onkológia. 61: 57-66. (2017).

5. Király AP; Alpár D; Fesüs V; **Marosvári D**; Matolcsy A; Bödör Cs. Az onkohematológia molekuláris diagnosztikai vizsgalómódszereinek alapjai. [Introduction to the molecular diagnostic methods of oncohematology]. Magyar onkológia. 60: 88-98. (2016).

6. Király PA; Kállay K; **Marosvári D**; Benyó G; Szőke A; Csomor J; Bödör Cs. Familiáris myelodysplasiás szindróma és akut myeloid leukaemia klinikai és genetikai háttere [Clinical and genetic background of familial myelodysplasia and acute myeloid leukemia]. Orvosi hetilap. 157: 283-289. (2016). **IF: 0.349**

7. **Marosvári D**; Alpár D; Király PA; Rajnai H; Reiniger L; Bödör Cs. A krónikus limfocitás leukémia genetikai háttere az újgenerációs szekvenálás korszakában [The genetic landscape of chronic lymphocytic leukemia]. Magyar onkológia. 60: 118-125. (2016).

8. Rajnics P; Kellner Á; Karádi É; Moizs M; Bödör Cs; Király PA; **Marosvári D**; Andrikovics H; Egyed M. Increased Lipocalin 2 level may have important role in thrombotic events in patients with polycythemia vera and essential thrombocythemia. Leukemia research. 48: 101-106. (2016). **IF: 2.501**

9. **Marosvári D**; Téglási V; Csala I; Marschalkó M; Bödör Cs; Timár B; Csomor J; Hársing J; Reiniger L. Altered MicroRNA Expression in Folliculotropic and Transformed Mycosis Fungoides. Pathology and oncology research. 21: 821-825. (2015). **IF: 1.940**