SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Bioinformatic and neuropathological analysis of *PARP1* and p53 pathway in gliomas

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BIOINFORMATIC AND NEUROPATHOLOGICAL ANALYSIS OF PARP1 AND THE P53 PATHWAY IN GLIOMAS

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The Examination takes place at the Library of the Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, on the 5th of January, 2018, at 11:00 AM.

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1. Introduction and literature review

Gliomas represent the most frequent primary tumours of the central nervous system. These tumours include clinically, histologically and molecularly different tumour types, and can be divided into well-circumscribed and infiltrating tumours. The first category includes the pilocytic astrocytoma (grade I), which is characterized by slow, non-invasive growth and considered as biologically benign. It manifests mainly in children or young adults. Infiltrating tumours include diffuse gliomas. The complete surgical resection of the these tumours is basically impossible due to their invasive growth. Nevertheless, the radio- and chemotherapy has proven to be effective. Based on the WHO classification gliomas can be divided into low-grade (grade II diffuse astrocytoma, oligodendroglioma and oligoastocytoma) and high-grade (grade III anaplastic astrocytoma, oligodendroglioma and oligoastocytoma, and grade IV glioblastoma (GBM)) tumours.

However GBMs share common histological features, they are individually different at the molecular level. Depending on the *IDH1/2* mutation status, the latest WHO classification distinguishes two subtypes of glioblastoma: IDH-wildtype and IDH-mutant GBMs. Furthermore, GBM can be also divided into four clinically relevant molecular subclasses based on their cytogenetic characteristics, copy number alterations, mutation status, and gene expression profiles: *Classical*, *Mesenchymal*, *Proneural* and *Neural*.

In the neuropathological diagnosis, the presence of $IDH1^{R132H}$ mutation, 1p/19q codeletion, and *ATRX* mutations may facilitate the isolation of diffuse gliomas. Among the listed markers, $IDH1^{R132H}$ mutation and the mutation status of *ATRX* can be accurately detected by immunohistochemistry (IHC), but 1p/19q codeletion detections are usually screened by fluorescent *in situ* hybridization (FISH). Based on literature data, the decreased expression of ATRX protein and p53 overexpression are mutually exclusive events with the 1p/19q codeletion. It can be assumed that the mutation status of *ATRX* and *TP53* may reflect the presence or absence of 1p/19q codeletion.

p53 protein is a nuclear transcription factor expressed in response to different stress signals such as DNA damage, heat shock, hypoxia, and oncogene overexpression. Activated p53 maintains the genome integrity by triggering cell-cycle arrest, DNA repair, and apoptosis. Contrary to other tumour suppressor genes, *TP53* alterations are usually missense producing stable full-length protein. Therefore, quick and accurate detection is an urgent task for the accurate diagnostic decisions and targeted therapies. Although the majority of *TP53* mutations may occur at any point in the gene, their primary localisation is in the 5-8 exons covering the

conserved DNA binding domain. *TP53* hot-spot mutations are represented in the 175, 245, 248, 249, 273 and 282 codons of the protein. Although DNA sequencing is the gold standard to identify *TP53* alterations, IHC has long been used as a surrogate method for mutation analysis in histopathological diagnostic practice, because overexpressed p53 protein indicates the presence of *TP53* alterations. Wild-type p53 is an unstable protein with a short half-life for its detection by immunohistochemistry, but mutant p53 can accumulate within tumour cells creating a stable target for IHC.

The biological basis of the resistance of tumours involves many factors, including molecular heterogeneity, and impaired DNA repair mechanisms. Currently, targeting the poly(ADP-ribose) polymerase 1 (PARP1) DNA repair protein in GBM is a new and promising aspect in clinical trials. PARP1 is a nuclear protein, and is normally involved in DNA repair and the maintenance of genomic integrity. PARP1 binds to DNA strand breaks and produces a poly (ADP-ribose) chain from NAD+ substrate which signals the cell to initiate DNA damage repair. The role of PARP1 has been already investigated in brain diseases, and its overexpression has been reported in various tumour types. Upregulated PARP1 can enhance the anti-apoptotic property of tumours resulting in resistance to DNA damaging agents. PARP inhibitors (PARPi) sensitize tumour cells to radiotherapy and to chemotherapeutic agents. The inhibitors attach to the catalytic domain of the protein thereby blocking synthesis of ADP-ribose polymers. Consequently, the cellular responses to DNA damage do not occur, leading to cell death.

Previous results are available for cerebral tumours with glioblastoma. Based on recent clinical trials, PARP1 DNA repair therapy may be a new and promising approach to glioblastoma. Due to overexpressed PARP1, inadequate DNA correction activity promotes anti-apoptotic properties of tumour cells, which may lead to chemotherapy resistance. Although the most important role of PARP1 is to improve single-stranded DNA breaks, it is likely to contribute to the induction of tumour progression. After DNA damage, PARP1, like p53, is one of the earliest expression proteins. In the cell with defective DNA, p53 undergoes various posttranslational modifications including PARP1 poly (ADP-ribosyl) ions. Additionally, endogenous PARP1 inhibits the transactivation function of p53, and PARP1 also regulates p53 responses to DNA damage. ADP-ribose polymers also play an important role in the binding ability of p53 DNA.

2. Aims

Despite extensive efforts to improve treatment, gliomas are still resistant to current therapies making them a compelling field of oncology. However, targeting the poly(ADP-ribose) polymerase 1 (PARP1) DNA repair protein in GBM is a novel and promising aspect in clinical trials, no comprehensive study has addressed *PARP1*'s expression characteristics and prognostic role regarding molecular heterogeneity in astrocytomas including GBM.

Identification of *TP53* mutations is part of the neuropathological diagnosis, helps in the isolation of astrocytic tumours, and also has prognostic value. Despite the controversial correlation between p53 overexpression and *TP53* mutations, p53 immunohistochemical detection of mutation status during routine (neuro)pathological diagnostics is a long-term method.

<u>The aims of recent work were:</u>

I. Study of histologically diagnosed glial tumours between 2007 and 2011 at our institution regarding the sex and the age of the patients and the WHO grades and the anatomical location of the tumours

II. Genomic characterization and prognostic role of *PARP1* in glioblastoma:
   - Determination of *PARP1* expression among glioma WHO grades and GBM molecular subtypes
   - Finding relationships between *PARP1* (mRNA expression, copy number alterations) and the mutation status of glioma markers (*ATRX*, *TP53*, *IDH1*)
   - Validation of the results of bioinformatic analysis by immunohistochemistry in an independent clinical cohort
   - Determining the exact relationship between PARP1 and p53 in glioblastoma

III. Analysis the frequency and immunohistochemical characteristics of somatic *TP53* mutations in brain tumours and other common tumour types.

3. Material and Methods

3.1 Genomic analysis of PARP1 in GBM

Genetic alterations of *PARP1*, somatic mutation, CNA, and mRNA expression (z-score, RNA Seq V2 RSEM) data were collected from two TCGA cohorts: Glioblastoma Multiforme (TCGA, Provisional) and Brain Lower Grade Glioma (TCGA, Provisional) using the cBioPortal for Cancer Genomics (www.cbioportal.org). Since the 'Glioblastoma Multiforme' dataset contains only WHO grade IV tumours, we also included the 'Brain Lower Grade Glioma' dataset in our analysis (containing genomic data of WHO grade II and grade III tumours). Therefore, using different cohorts of patients allows us to examine the significance of *PARP1* in malignant transformation of lower grade astrocytomas to glioblastoma. Clinicopathological data for each patient, including: age, sex, and survival time, was compiled from the TCGA portal (www.tcga-data.nci.nih.gov) and tabulated with genetic data. Cases for the analysis were selected according to the following criteria: only patients older than 18 years with available clinical data were included and oligodendrogliomas such as mixed gliomas were omitted. Only tumours with complete genomic data (available somatic mutations, CNA, and mRNA expression information) were selected for the *PARP1* mRNA expression analysis. Altogether, 135 WHO grade IV glioblastoma and 96 lower grade astrocytomas (grade II & grade III) were assembled for the present analyses. GBM subtypes were accessed on 119 cases, with the following distribution: 57 *Mesenchymal*, 43 *Classical*, 16 *Proneural*, and 5 *Neural*. The relationships between *PARP1* mRNA expression and the overall patient survival were analysed by dividing the samples into PARP1-low and PARP1-high expression groups, based on median mRNA expression z-score in TCGA dataset.

3.2 Patients

In our retrospective study, gliomas had been histologically diagnosed at our institute between 2007 and 2011 were analysed: 127 grade II. gliomas (62 male/65 female) and 214 grade III. and IV gliomas (110 male/104 female). Retrospective selection of the previously histologically diagnosed samples was conducted using the *eMedSolution* database. Cases for the analysis were selected according to the following criteria: WHO grade, sex and age of patients, and anatomical location of tumours.

For testing bioinformatic results, samples were obtained from 60 patients (30 males and 30 female) diagnosed with glioblastoma between 2006 and 2014 at our institution. The mean

age at diagnosis was 58.47 ± 9.03 years (range 30.21- 76.67 years). After surgical removal, sections were cut and stained with haematoxylin-eosin (H&E) from formalin-fixed and paraffin-embedded (FFPE) blocks. All the histopathological specimens were reviewed and diagnosed by a neuropathologist according to the recent *'WHO Classification of Tumours of the Central Nervous System'* criteria.

3.3 Immunohistochemistry

Immunohistochemical staining was performed on 4-μm-thick FFPE sections according to the manufacturers' protocols. At first, sections were deparaffinised in xylene and rehydrated in a graded series of ethanol. Heat-induced epitope retrieval was performed utilizing citrate buffer (pH 6.0). After blocking endogenous peroxidase activity, sections were incubated with primary antibodies (anti-p53, anti-ATRX, anti-$IDH1^{R132}$, and anti-PARP1) for 6 hours at room temperature. Visualization was achieved with SuperSensitive™ One-step Polymer-HRP Detection System on Leica Bond Max™ automated IHC stainer, employing 3,3'-diaminobenzidine (DAB) followed by hematoxylin counterstaining. Adequate positive and negative controls (with the omission of the primary antibodies) were included in the immunohistochemical experiments. Cases with ≥10 % stained cells were defined as positive for $IDH1^{R132}$ and p53. A cut-off point of 10 % was considered for the evaluation of presence or absence of nuclear ATRX. All FFPE sections were scored in a blinded manner by at least two independent observers. The expression of all IHC markers was determined semi-quantitatively. Ten randomly selected high-power fields. The PARP1 expression was evaluated based on the distribution of positive cells: "0" (<5%, negative), "1" (5–25%, sporadic), "2" (25–50%, focal), or "3" (>50%, diffuse). For the statistical analysis, negative and sporadic staining was combined as low PARP1 staining and focal and diffuse nuclear staining as high PARP1 staining.

3.4 Investigation of the IHC features of TP53 mutations

In recent work the (R17) dataset of somatic *TP53* mutations (http://p53.iarc.fr/TP53SomaticMutations.aspx) was used for the characterization of *TP53* mutations, and correlate them with IHC expression data. The description of *TP53* mutations is at the protein level according to the Human Genome Variation Society (HGVS) standards, using the P04637 Uniprot reference sequence. *TP53* mutations with known IHC data were retrieved and the expression features were analysed in different aspects: I) the localization within the

TP53 gene; II) the effects of the mutation; III) mutation types; and IV) the affected structural motifs of p53 protein. Based on these characteristics, we divided *TP53* somatic mutations into three groups: Group A (strongly IHC positive, hot-spot mutations; mutation frequency ≥ 0.013 and IHC positivity ≥ 0.85), Group B (mainly IHC negative nonsense mutations; mutation frequency ≤ 0.011 and IHC positivity ≤ 0.44), and Group C (IHC positive, non-frequent missense mutations; mutation frequency ≤ 0.072 and IHC positivity ≥ 0.74). Only mutations with 15 or more IHC results were involved in the classification.

3.5 Statistical and bioinformatics analysis

Statistical analyses were performed using R (http://www.r-project.org) and SPSS 21.0 softwares (SPSS Inc., IL, USA). Comparisons between groups were performed with Pearson's Chi-square ($\chi 2$) test for categorical variables. Age of onset associations between groups were done with the Mann-Whitney U test. The association between mRNA levels was measured using the Kendall's tau and Spearmann's correlation test. The difference in mRNA expression levels, between groups was calculated using a two-tailed Student's t-test. Kaplan–Meier method and Log-Rank (Mantel-Cox) test were used for overall survival measurements. Two-sided tests were computed and statistical significance was set at $p<0.05$. Gene-gene interaction network to demonstrate p53 pathway and PARP1 interaction was generated by the GeneMania Cytoscape 3.4.0 application. Physical, co-expression and gene-gene interactions were evaluated. The Gene-E version 3.0.204 (http://www.broadinstitute.org/cancer/software/GENE-E/index.html) software was used to depict heat map differences between *TP53* mutations for tumour types.

4. Results

4.1 Characterization of the histologically diagnosed diffuse gliomas

In the first step of our work, a total of 127 low (II) and 214 high (III and IV) grade glial brain tumours were studied by the date of the diagnosis, WHO grades, sex, age and the anatomical localization of the tumours. Of 127 cases 51 (40%) were diffuse astrocytoma, 39 (31%) were oligodendroglioma and 37 (29%) were. The mean age of patients (62 male and 65 female) was 39 years (± 20.3). The mean age of patients diagnosed with diffuse astrocytoma was 37.6 years, with oligodendroglioma 34.8 years and 45.5 years with oligoastocytoma. Male and female sex ratios were 1:1.3 in diffuse astrocytoma, 2:1 in oligodendroglioma was and 1:1.6 in oligoastocytoma patients.

Totally 85% of high-grade grade tumours (182 cases) WHO grade IV. glioblastoma, while the remaining 15% (32 cases) grade III. anaplastic gliomas. Of the 184 glioblastoma cases, primary glioblastoma was diagnosed in 142 patients (78%), secondary glioblastomas were diagnosed in 24 (13.2%), giant cell glioblastoma and gliosarcoma were reported in 8-8 (4.4-4.4%) cases. Of 32 grade III. tumours 19 patients (59.4%) had anaplastic astrocytoma, 8 (25%) patients had anaplastic oligodendroglioma and 5 (15.6%) anaplastic oligoastocytoma. The mean age of patients with high-grade gliomas was 57 years (± 16.4) and the sex ratio was 110 male and 104 female. More particularly, glioblastoma was diagnosed in 88 male and 94 female whereas anaplastic glioma was diagnosed in 22 male and 10 female. The anatomical localization of grade II. tumours was available in 74 cases. The tumours were localised in the frontal lobe in case of 41 patients, in the temporal lobe in 22 cases, in the lumbar lobe in 5 cases, in the brain stem in 4 cases, in the cerebellum in 2 cases and 1 case was found in the parietal lobe. The most frequented localisation for astrocytic tumours (n = 24) was the frontal lobe (54%), the temporal lobe (25%), the brain stem (8%), and parietal and occipital lobes and cerebellum (4-4%). The localization of oligodendroglial tumours (n = 25) was nearly identical in the frontal and temporal lobes (48 vs. 40%), and infrequent in the the brain stem (8%) and in occipital lobe (2%). The majority (60%) of oligoastocytomas (n = 25) were identified in the frontal lobe, 24% in the temporal lobe and 12% of the tumours was found in the occipital lobe. Anatomical localization of tumours was available in 91 glioblastoma cases. These tumours had been localised in the frontal lobes (n = 51), the temporal lobes (n = 25), the parietal lobes (n = 11), the occipital lobes (n = 3). Considering the anatomical localisation of classical glioblastoma (n=77) it was more frequent in the frontal lobe (53%) followed by the temporal lobe (28%), the parietal lobe (14%) and the occipital lobe (4%). In one case the tumour was localised in the

cerebellum. Gliosarcoma (n=6) occurred in the frontal and temporal lobes in 3 to 3 cases, while most giant cell GBM (n = 7) was localized in the frontal lobe and in the temporal lobe in one case.

4.2 Genomic and transcriptomic characteristics of PARP1 in GBM

We next analysed *PARP1* mutations and CNAs of GBM samples in the TCGA database via cBioPortal (http://www.cbioportal.org/public-portal/). Among the 135 GBM cases, only two *PARP1* somatic mutations were observed (V948I and A709T). Information on CNA data was available for 562 GBM samples. *PARP1* exhibited a low-level gain and heterozygous deletions in more than 14% and 6% of the cases, respectively. On the other hand, *PARP1* amplification was infrequent (0.35%), and homozygous deletion was not observed. Next, we examined the relative *PARP1* mRNA levels in GBM. The database included 135 adult GBM cases with DNA sequencing, CNAs and mRNA expression data according to the screening criteria. Lastly, for determining the possible regulation mechanisms associated with *PARP1* expression values, we evaluated the correlation between CNAs and mRNA levels. A significant expression difference was found between heterozygous deletion and diploid ($p=0.005$), gain and diploid ($p<0.001$), as well as heterozygous deletion and gain statuses ($p<0.001$). These results demonstrate a close link between the copy number and gene expression in glioblastoma.

To determine the relevance of *PARP1* in malignant transformation of astrocytomas, the Brain Lower Grade Glioma (TCGA, Provisional) dataset was investigated through cBioPortal. Altogether, CNA data of 169 lower grade tumours (55 grade II and 114 grade III astrocytomas) were added to the 562 GBM samples. We found, that grade II tumours have the lowest CNA rate (0.036) followed by grade III astrocytomas (0.167) and grade IV GBM (0.215), and high *PARP1* copy numbers correlate with higher histological grades ($p<0.001$). Homozygous deletions occurred in only 0.1% of grade II tumours. *PARP1* amplification was also rare, found in only 0.3% of GBMs. In total, mRNA expression data were available for 97 lower-grade astrocytomas (29 grade II and 67 grade III tumours). Increased *PARP1* mRNA levels were found in the high-grade tumours as opposed to grade II astrocytomas (GII vs. GIII, $p=0.003$, and GII vs. GIV, $p<0.001$). Although *PARP1* expression was increased in both grade III and IV tumours, there were no significant changes between those two grades. *PARP1* CNAs showed significant differences in distribution among WHO grades ($p<0.001$).

PARP1 expression signature was also analysed in the context of the genetic heterogeneity of GBM. We evaluated the association between *PARP1* expression and the

mutation status of *IDH1*, *ATRX* and *TP53* genes. We observed that samples carrying mutated *ATRX* (p=0.006) and *TP53* (p=0.015) were associated with increased *PARP1* levels. The *IDH* status of GBMs did not show any association with *PARP1* expression. Since the TCGA dataset contains only the *IDH* and *ATRX* status of the samples, therefore all cases were also classified into wildtype (n=92) and mutant *TP53* (n=43) tumours. Next, mutant *TP53* samples were further divided into missense (n=33) and null (n=10) mutations in order to investigate the mutation effects on *PARP1* expressions. *PARP1* mRNA expression was higher in *TP53* mutated cases (p=0.015) than in its wildtype counterpart. Importantly, there was no significant changes between missense and null *TP53* mutations.

Due to the results of the bioinformatics analysis, where *PARP1* mRNA expression had a significant correlation with *TP53* and *ATRX* mutational status, the markers were also examined by immunohistochemistry. Ninety percent (54/60) of all cases were PARP1 positive, while 10% (6/60) were negative by IHC. In our clinical cohort, PARP1 IHC expression was significantly associated with the expression of p53 (p=0.0281) and *ATRX* (p=0.002) but not with that of *IDH1*.

In the exploration of the subtype-specific role of *PARP1* in GBM, we identified that *Proneural* and *Classical* subtypes showed an increased *PARP1* expression. More specifically, *Proneural* (mean z-score: 0.801) and *Classical* (mean z-score: 0.375) subtypes have the highest average *PARP1* mRNA level, followed by *Mesenchymal* (mean z-score: -0.121) and *Neural* (mean z-score: -1.049), respectively. Simultaneously, we also assessed whether *PARP1* CNAs were related to specific GBM subtypes. CNAs information on transcriptional subtypes were available for 475 cases. We found that the *Proneural* subtype had the highest CNA rate (0.275), followed by the *Mesenchymal* (0.226), *Neural* (0.224), and *Classical* (0.129) subtypes. *PARP1* copy number gains were observed in all subtypes: *Mesenchymal* (4.4%), *Proneural* (4.4%), *Classical* (2.9%) and *Neural* (2.3%). Heterozygous deletion of *PARP1* was found in 3.4% of *Mesenchymal* GBMs. PARP1 amplification occurred only in *Classical* and *Mesenchymal* subtypes (0.2-0.2%).

To assess the clinical relevance of *PARP1* in GBM, we examined the association between *PARP1* mRNA expression and the overall survival in TCGA GBM samples. The mean overall survival (OS) of the TCGA patients was 13.3 months. The 135 GBM cases were assigned into PARP1-high (n=68) and PARP1-low (n=67) groups, using median *PARP1* mRNA expression z-score as cut off value. Although patients with PARP1-low expression had longer survival, there were no statistically significant differences observed between the groups. The mean OS of TCGA patients across different GBM subtypes was the following: *Classical*

=12.7; *Proneural* =19.8; *Mesenchymal*=11.7, and *Neural* =7.7 months. Kaplan-Meier survival analysis indicated that patients of the PARP1-low group had statistically significant shorter overall survival time compared with their PARP1-high counterpart (p=0.031). No significant differences were noted in the survival values between the PARP1-low, and -high expression cohorts of other GBM subtypes. We also investigated whether *PARP1*'s genetic signatures were related to the clinicopathological characteristics of GBM patients. No significant associations were observed between *PARP1* status and age and sex. We analysed cumulative effects of p53 and *PARP1* on survival with GBM. TCGA samples were further classified according to their *PARP1* levels and *TP53* mutation status: I) $TP53^{mut}$/PARP1-high (n=29); II) $TP53^{mut}$/PARP1-low (n=14); III) $TP53^{WT}$/PARP1-high (n=39); and IV) $TP53^{WT}$/PARP1-low (n=53). We found, that GBM patients with wild-type *TP53* gene and PARP1-high level have a shorter survival (p=0.039) compared to the other groups.

To extend our investigations, the relationship between *PARP1* status and the p53 pathway (*CDKN1A* (p21), *MDM2*, *MDM4*, *CDKN2A* (p14), and *TP53BP1*) was also examined. Regarding mRNA expressions, *PARP1* displayed significant correlation with *TP53*, *CDKN1A*, and *TP53BP1*. Accordingly, mRNA expression levels of *TP53* (p=0.003), and *TP53BP1* (p=0.004) were increased in PARP1-high, and *CDKN1A* (p=0.009) was increased in PARP1-low GBMs. *PARP1* levels showed correlation with copy number alterations of *CDKN2A* and *MDM4* genes. More specifically, *PARP1* levels were decreased in homozygously deleted *CDKN2A* (p=0.013) cases, and increased in cases with *MDM4* gain (p=0.026) when we compared CNAs to the diploid variants of the genes. However, there was no significant association between *MDM2* CNAs and *PARP1*.

4.3 Immunohistochemical correlates of TP53 somatic mutations in gliomas and other tumour types

A key aim of this part of our work was to provide a complex characterisation of IHC features of *TP53* mutations. p53 IHC data was available in 7878 mutations, representing 26.5% of the 29711 *TP53* somatic mutations in the IARC Database. The IHC staining was positive in 6026 mutations (76.5%), whereas 1852 mutations (23.5%) were negative by IHC (p<0.001). Regarding mutation types, single nucleotide alterations were predominantly IHC positive at a range between 74.9% and 84.6%; tandem (93.8%) and complex mutations were also positive, whereas deletions and insertions were negative (57% and 59.7%, respectively). Almost 88% of

missense mutations were p53 IHC positive, whereas 71.2% of nonsense mutations were negative for IHC ($p<0.001$).

Almost 80% of the mutations were positive within the coding regions, whereas the majority of mutations in the non-coding sequences were IHC negative (56.5%) ($p = 0.001$). Mutations outside and within the CpG island regions were positive in 83.3% and 73.3%, respectively ($p < 0.001$). *TP53* alterations were positive in non-splice site sequences as well as in alternative and cryptic sites (77%, 83.1% and 74.2%, respectively), but 64.3% of the mutations were negative in the consensus splice sites. The IHC expression patterns were diverse among the structural motifs of mutated p53.

Mutations were unequivocally positive in L1/S/H2 (86.2%), L2/L3 loops (84.3%), and NDBL/beta-sheets motifs (70.1%). Furthermore, alterations were positive in the C-terminal (55.6%), C-terminal/NLS (58.1%), and N-terminal/Transactivation/NES (60%) motifs. On the other hand, *TP53* mutations were negative in the N-terminal (76.5%), N-terminal/Transactivation (60.6%), and SH3-like/Proline-rich (59.4%) motifs. The structural motifs of 224 mutations were unknown.

In our study, IHC expression profile was determined for each *TP53* mutation and compared to the clinical pathology characteristics. In the next step, the frequency of the *TP53* mutations in the IARC database was determined and then the IHC positivity (IHC positive cases/all cases) was assigned to each mutation. The majority of the mutations in hotspot p53 codons were strongly positive: 175 (89.7%), 245 (90.7%), 248 (93.3%), 249 (88.0%), 273 (92.6%) and 282 (90.9%). In contrast, mutations were usually negative among the frequently altered codons like 213 (57.7%), 196 (65.2%), 306 (60.9%), 146 (63.6%) and 298 (60.7%). All mutations within the hotspot codons (R175H, G245S/D, R248Q/W/L, R249S, R273H/C/L, and R282W) were positive for p53 IHC at a range of 87.2-100%. Contrary, frequent nonsense mutations such as R213*, R196*, R306*, W146*, and E298* were not able to be detected (63.4-75.6%) by IHC. Based on the mutation frequencies and IHC positivity, *TP53* mutations possess more than 15 IHC data ($n = 78$) were categorized into three distinct groups. Hot-spot and IHC positive mutations (9/78; 11.5%) were in *Group A*, frequent and mostly IHC negative mutations (7/78; 9%) were in *Group B*, whereas infrequent and IHC positive mutations were in *Group C* (62/78; 79.5%). Principal components analysis of the groups by two components also confirmed differences between the three groups. All alterations in *Group A* and *C* were missense, whereas in *Group B* they were nonsense ($p < 0.001$). The majority of *TP53* mutations in *Group A* and *B* localized within exons 5-8 ($p<0.001$), and they commonly occurred in the CpG sequences ($p<0.001$).

The p53 IHC was predominantly positive (75.8% vs. 76.4%) in both sex but females with IHC negative mutations were significantly younger (55.90 ± 15.391 vs. 58.57 ± 15.062 years, p=0.0182). There was no significant difference regarding the mean age (56.44 ± 16.86 vs. 57.22 ± 15.772, p=0.204). *TP53* alterations were IHC positive in almost all human cancers in the database at a range between 50 and 100%.

Sex distribution and the mean age at diagnosis were also analysed in the p53 IHC groups. All group represented a female dominance (p=0.0042). Significant differences were observed in five tumour sites (breast, bladder, haematopoietic system, liver, head and neck) in term of the age at diagnosis among the IHC groups. Breast cancer patients carrying nonsense mutations (*Group B*) were significantly younger (48.83±13.69 years) than patient in the other groups *(Group A*: 55.06±13.99 years *& Group C*: 55.97±14.07 years). Similar difference was observed in bladder cancer, but patients were notable younger in only *Group B* (59.52±14.20 years) compared to *Group A* (64.44±12.57 years). Contrary, liver carcinoma patients in Group A were significantly older then in *Group C*. Comparison of the *Group A* and *C* revealed that patients with frequent missense mutations were younger with haematopoietic system, but older with head and neck tumours. Nonsense *TP53* mutations (*Group B*) have a worse prognosis and shorter survival. The majority of the alterations proved to be IHC negative, therefore we analyzed *Group B* in detail. Considering the frequency of occurrence of mutations, significant differences were observed between different tumour types.

IHC characteristics of somatic mutations of *TP53* were available 353 gliomas. 84% of the mutations described in the gliomas were IHC positive. Since the IHC profile of the wild-type *TP53* gene is not available in the IARC TP53 database, we could not test the false positive IHC stainings. Therefore, our study was supplemented with the *TP53* mutation status of 232 astrocytomas (II, III and IV) in the TCGA database and compared with their mRNA expression. Regarding the characteristics of the mutations, 22.2% of the mutations were null/nonsense mutations mostly located in the introns. Based on the mRNA expression / mutation status correlation, the mean mRNA expressions (z-score) regarding the *TP53* status were the following: wild type (-0.004); missense mutations (0.08); nonsense mutations (-0.46). Compared to the other tumour types, *Group B* nonsense *TP53* mutations with IHC negative staining are less common among brain tumours.

5. Discussion

However glioblastoma is one of the most intensively studied tumour, there is no detailed information available on the occurrence of gliomas in Hungary. One of the aims of our work was to replace this deficiency, therefore we analysed the histological diagnosed 341 glioma cases between 2007 and 2011 at our institution. Tumours were also analyzed for the date of diagnosis, WHO grades of the tumours, sex, age and anatomical localization of tumours.

However glioblastoma is one of the most intensively studied tumour, yet the majority of patients are still resistant to the conventional therapies. Thus, the identification of novel biomarkers to improve the management of these tumours is an ongoing and challenging task in the field of oncology. Although PARP1 inhibition is a potential therapeutic target in gliomas, its efficiency in the context of heterogeneity is unknown. It had been described, that PARP1 is overexpressed in a variety of cancers, including glioblastoma. Increased *PARP1* expression has been reported in paediatric high-grade astrocytomas, medulloblastoma, and ependymoma. Furthermore, in GBM stem cells, the combination of PARPi and temozolomide (TMZ) may represent a valuable strategy to reverse the stem cells' chemo-resistance. According to a recent study, PARPi together with TMZ can exert synergistic anti-tumour effects in glioma lines. Moreover, the dose reduction of TMZ is associated with the sensitivity of each cell line to PARPi as single agent. Recent bioinformatic analysis of TCGA datasets revealed that PARP1 expression is restricted to higher grade tumours, and partially caused by genomic gain. Our findings underline that *PARP1* is a marker candidate of higher-grade in astrocytomas, presumably because higher *PARP1* expression facilitates the repair of damaged DNA and, thereby, overcomes the genetic instability characteristics of tumour cells.

Given the heterogeneous landscape of GBM, it is relevant to investigate *PARP1*'s associations with key molecular markers. The importance of *ATRX*, *IDH1*, and *TP53* mutations in the early development and the progression of astrocytic glioma lineage is well-known. These markers also have treatment and prognostic relevance and their mutation status can distinguish astrocytomas from oligodendrogliomas, as well as secondary from primary GBM. *PARP1* expression can occur in both IDH-wild type and mutated GBMs. Although there was no statistically proven association between *PARP1* expression and *IDH* mutation status, a recent study reported that 2-hydroxyglutarate produced by mutated IDH induces PARP inhibitor sensitivity in patient-derived primary glioma cells and genetically matched tumour xenografts, which has therapeutic relevance. Increased *PARP1* expression levels were associated with

ATRX and *TP53* mutations. *ATRX* alterations occur in the vast majority of lower-grade astrocytomas and IDH1-mutated (secondary) GBMs. Somatic *TP53* mutations play important roles in gliomas, particularly in the tumourigenesis of lower grade astrocytomas and IDH1-mutated GBMs. Considering that the inhibition of PARP1 enzyme is dose-dependent, our results indicate that PARP inhibitors could be more effective in *ATRX* and *TP53* mutated tumours, where *PARP1* levels are usually increased.

Although high-throughput technologies are widely used for diagnostic purposes, there is still a need to improve the IHC-based stratification of GBM with the integration of molecular data. The IHC detection of key molecular markers is possible and highly informative: I) using $IDH1^{R132H}$ mutation specific antibody; II) most ATRX mutations result in undetectable ATRX expression by IHC; and III) mutated p53 protein accumulates in the nucleus of tumour cells. Furthermore, we have demonstrated that IHC expression of PARP1 has an inverse correlation with ATRX, and linear correlation with p53 staining. These observations suggest that PARP1 IHC expression along with p53 overexpression and ATRX loss can be promising predictive markers for PARPi in GBM.

Our study investigates the genomic signature and prognostic significance of PARP1 in GBM subtypes. We present evidence, that *PARP1* can distinguish *Proneural* and *Classical* from the other subtypes as increased *PARP1* expression was increased in these two subtypes. We observed that high *PARP1* mRNA expressions were associated with shorter survival in the *Classical* group. It was previously presented that *Classical* GBM is characterized by *EGFR* amplification and wild-type *TP53*, whereas *Proneural* is characterized by both *IDH1* and *TP53* mutations and *PDGFRA* amplification. These results indicate that PARP1 and p53 are suitable markers to distinguish *Proneural* and *Classical* subtypes, and have also a prognostic relevance in GBM.

We found that PARP1 IHC expression is correlated with p53 positive cases. In addition, *PAPR1* mRNA levels were higher in samples with *TP53* mutations. Importantly, the detection of *TP53* nonsense mutations is not accurate by IHC due to the truncated protein. Beside the increased *PARP1* levels in *TP53* mutated cases, there was no statistical difference between missense and null mutated samples.

Both *PARP1* and p53 play important roles in maintaining genomic integrity. Several studies have shown that genomic instability is usually correlated with poor prognosis. It has been previously reported that high level of *PARP1* expression is often associated with poor overall survival in cancer. Although there is a trend towards shorter survival of PARP1-high patients as compared to PARP1-low patients, no significant differences were observed when all

GBMs were considered. Interestingly, we found a significantly shorter survival in PARP-high patients with wildtype *TP53* gene – possibly because not only *TP53* mutations but also an impaired p53 pathway can facilitate tumour progression. The p53 signalling pathway mediates several cellular processes including growth arrest, angiogenesis, apoptosis, and DNA repair. It is widely accepted, that the progression and recurrence of glioblastoma is related to p53 pathway abnormalities in 87% of primary GBM. To gain insights into the interaction between p53 and *PARP1*, we investigated and found that *PARP1* showed association with *CDKN2A* deletions and *MDM4* gain. These two genetic alterations are frequent in GBM, and result in abnormal p53 signalling in tumour cells. Furthermore, *PARP1* was negatively correlated with *CDKN1A*, which is a downstream member of the p53 cascade involved in the p53-mediated G1 arrest. This observation suggests that down-regulated *CDKN1A* cannot inhibit the Cyclin E-CDK2 complex in GBMs with higher *PARP1* levels. Consequently, it can result in an augmented cell-cycle activity and absence of apoptosis. Furthermore, it has been reported, that low levels of 53BP1 could predict resistance to PARP inhibitors, because 53BP1 depletion reduces the cytotoxicity of PARP inhibitors. Consistent with this notion, we found an overall low expression of *TP53BP1* in GBM with higher levels in high-PARP1 cases.

The relationship between *TP53* mutations and p53 nuclear accumulation has not yet been clarified. Although routine diagnostic processing, immunohistochemistry is a frequently used technique for *TP53* mutational analysis, its reliability can be argued for false positive and negative cases. The aim of our study was to evaluate the IHC expression pattern of somatic mutations of *TP53* in different tumour types and to find correlation with their clinical pathophysiological characteristics. Therefore, we made a systematic analysis of the R17 dataset in the IARC TP53 database. Publicly available tumour profiling and data sequencing databases allow more complex research, including molecular epidemiology, clinical surveys, and structural analysis. The IARC TP53 Mutation Database (http://p53.iarc.fr/) contains data on prevalence and pattern of more than 28000 somatic mutations in human cancer, tumour phenotype annotations, clinical parameters of patients, structural and functional influences, and IHC patterns of mutations. The database includes all the described *TP53* mutations, which were sequenced, published in professionally reviewed journals or edited in mutation repositories. Under normal conditions, the synthesis and degradation of p53 is strictly regulated, its expression level is very low. The longer half-life of aberrant p53 results in an overproducing protein that can be detected by immunohistochemistry. Our study confirms that more than 75% of *TP53* mutations are uniformly associated with IHC-detectable p53 accumulation. This total

positivity was observed in all tumour sites. It is opposed to other tumour suppressor genes, where protein expression is reduced or absent due to deletions and nonsense mutations.

Most *TP53* mutations occur predominantly in the DNA binding domain (94-292 side chains), and our results show that more than 94% of IHC positive modalities are found in this domain. *TP53* mutations formed in this domain may lead to the most aggressive clinical phenotype, which presumably reduces the protein's biological activity, mutations in these critical motives are mostly IHC positive and can therefore be well detected during diagnosis. With regard to the structure of the gene itself, the majority of the mutations occur in 5-8 exons, more than 95% of these mutations are found in these exons that exhibit IHC positivity. Point mutations in intracellar *TP53* may eliminate p53 functions. Our observations confirm earlier reports, as 87% of IHC-detecting mutations were bases-related substitutions in the coding regions. These mission mutations generally result in full length protein and more than 80% IHC positive according to the IARC database. The rare occurrence of duplication and complex *TP53* mutations in human tumours were also IHC positive. In the *TP53* gene splice mutations are also rare events. Accordingly, only 3% of mutations develop in conserved dinucleotides, including splice sites with varying distribution and IHC characteristics. The transcribed protein is stable enough and not degraded. Splice variants exhibit IHC positivity in alternate and hidden splice acceptor sites; consensus splice sites were negative. There was no significant difference in IHC expression within or without CpG sequences, both of which were mostly positive (83.3% and 73.3% respectively).

Somatic *TP53* mutations were classified into three groups based on their frequency and IHC positivity. Hot spot mutations (*Group A*) and common nonsense mutations (*Group B*) form a separate group, while rare, highly positive missense mutations have been introduced into *Group C*. The starting point of our study was that the IHC labeling of nonsense *TP53* mutations (*Group B*) is impossible because of the absence of a gene product. These truncating mutations are common in younger age-related diseases where the *NF2* and *HNF1A* genes are affected. Females with IHC negative mutations were younger. According to our classification of TP53 mutations, the age at diagnosis of Group B was lower in breast and bladder cancers, and interestingly, it was higher in tumours of the haematopoietic system compared to Group A and C. The frequency of R213* and Q192* was also increased in breast, as well as R306* and Q192* mutations frequency in bladder cancer. In breast cancer, R213* TP53 mutations are more frequent in the basal-like subtype. In colon cancer, R196*, R213* and R306* mutations were also common. Furthermore, R213* mutation was common in colorectum, and R306* in rectum carcinomas. It was previously reported, that R306*mutation was present in 15.27% and

39.5% of alleles in the invasive component of the primary tumour and in the metastasis, respectively. The W146* mutation was slightly more frequent in skin and mouth cancers in our analysis. Although nonsense (Group B) mutations are infrequent, they indicate worse overall survival when p53 is truncated. It was previously showed, that *TP53* null mutations presumably related to recurrent tumours, indicating that deleterious alteration confers an increased and earlier probability for recurrence. Furthermore, tumours with nonsense (*Group B*) mutations are more likely to develop metastatic tumours compared to those cancers that contain either missense mutations (*Group A & C*) or are wild-type p53. Patients with nonsense *TP53* alterations have an increased risk to have more vascular tumours. In our study, more than half of the alterations (56.5%) in the non-coding regions were mostly negative by IHC. We observed that *TP53* mutations with negative IHC results were most frequently caused by deletions or insertions. Drugs inducing the read-through of early stop codons caused by mutations could be a promising therapeutic strategy of the cancer linked with non-sense mutations in tumour suppressor genes. Aminoglycoside antibiotics such as gentamicin and G418 can promote premature termination codon read-through results in the partial restoration of full-length protein. According to a study Q192*, R213* and E298* TP53 mutations displaying high induced read-through level. The aminoglycoside treatment strongly and specifically stabilized mutant p53 mRNAs. Although induction of read-through of premature stop codons is effective, the clinical use of these agents is still limited by their toxicity.

There is still no consensus as to which antibodies are most appropriate for evaluating mutation-associated p53 expression. None of the routinely used p53 antibodies (CM1, Pab1801, DO1 and DO7) differentiate between mutant and wild-type p53 proteins. While, CM1 antibody binds to the full length protein, DO7, DO1 and Pab1801 recognize epitopes only in the N-terminus of the human p53 protein. Importantly, the most commonly used DO7 antibody can detect only truncation mutations in exons 9-10. A further limitation of p53 IHC is that not only *TP53* mutation but also disturbed p53 pathway can result in abnormal p53 expression. Therefore, amplification of *MDM2* or *MDM4* as well as repressed $p14^{ARF}$ or *TP53* by promoter methylation can cause reduced p53 expression resulting in a limited sensitivity of IHC. Intensive efforts have been made to improve the reliability of p53 IHC as a surrogate method. Combined usage of antibodies that target various p53 epitopes or p53-related proteins, as well as quantitative scoring methods seem to be valuable approaches for *TP53* mutation prediction. A study using eight antibodies relating to p53 stabilization and transcriptional activation described that i) overexpressed p53 without increased MDM2 indicates inactivating mutations that stabilize p53; ii) tumours with overexpressed p53 and concurrent increase of MDM2 do

not have p53 mutation. iii) phosphorylated p53 expression correlates with total p53 levels and iv) does not predict *TP53* mutation status. Nevertheless there are no subsequent studies confirming these findings. An antibody cocktail of DO1 and DO7 antibodies could identify 93% of cell lines and patient samples with *TP53* missense mutations in the exons 5 to 8 region in prostatic adenocarcinoma. Combined IHC of PLK1 (Polo-like kinase-1), p21, and p53 is slightly more sensitive for predicting *TP53* status and may facilitate differentiation of missense and nonsense mutations. The p21 is a transcriptional target of p53, therefore its expression is used for decreasing false positivity of p53 IHC. IHC positivity of PLK1 along with negative p53 IHC can reflect nonsense *TP53* mutations and may decrease the possibility of false negative IHC, because mutant p53 fails to repress PLK1 expression. A study using p53 antibodies like DO7, DO1, and E26 and tagged-amplicon next generation sequencing of *TP53* in high-grade serous ovarian carcinomas and endometrioid carcinomas demonstrated that optimized p53 IHC assay is a useful surrogate for the *TP53* mutation status, and that combination of p53 IHC and sequencing should be the gold standard in assessing the p53 functional status for clinical trial inclusion. IHC scoring may correlate with *TP53* mutations and could increase the accuracy of p53 IHC. In a recent study the missense *TP53* mutations have high expression of p53, whereas low expression was associated with non-missense mutations (i.e. frameshift, in-frame, nonsense, and splice). Furthermore, wild-type *TP53* tumours displayed intermediate p53 IHC expression. A limitation of our analysis is that we cannot predict the overall, and tumour specific sensitivity of p53 immunohistochemistry because the IARC TP53 Database does not contain IHC results of wild-type p53 expression. Consequently, the analysis and exclusion of false positive cases has not been possible. IHC antibodies are not listed in the database, therefore their specificity and association with somatic mutations could not be analysed.In conclusion, the majority of *TP53* mutations were missense and IHC positive, whereas most nonsense and frameshift mutations and deletions were immunonegative. Significant correlations were observed between the age at diagnosis and the immunohistochemical patterns of *TP53* mutations in breast, head & neck, bladder, liver and haematopoietic cancers. In certain tumour sites there is an increased likelihood of false negative IHC associated with rare nonsense mutations. Our frequency- and immunopositivity-based classification is useful in patient stratification and has prognostic implication.

6. Summary

Gliomas represent the most frequent brain tumour in adult. Despite the efforts to develop novel treatments, GBM is still highly resistant to current therapies therefore new diagnostic approaches as well as effective therapies are needed to improve patient outcome.

At present, no detailed data are available regarding the status of gliomas in Hungary. In view of this deficiency, in the early stages of our work, we examined histologically diagnosed glial tumours between 2007 and 2011. In our study, 341 glial tumours were characterized and grouped according to their frequency, sex and age of patients, and anatomical localization of tumours.

Although PARP1 inhibition is a promising therapeutic target, no comprehensive study has addressed *PARP1*'s expression characteristics and prognostic role regarding molecular heterogeneity in astrocytomas including GBM. Our aim was to evaluate *PARP1*'s associations with survival, WHO grade, lineage specific markers, and GBM transcriptomic subtypes. We demonstrated that PARP1 gain and increased mRNA expression are characteristics of high-grade astrocytomas, particularly of *Proneural* and *Classical* GBM subtypes. Additionally, higher *PARP1* levels exhibited an inverse correlation with patient survival ($p<0.005$) in the *Classical* subgroup. *ATRX* ($p=0.006$), and *TP53* ($p=0.015$) mutations were associated with increased *PARP1* expression and PARP1 protein level correlated with ATRX loss and p53 overexpression. Furthermore, higher *PARP1* expression together with wildtype *TP53* indicated shorter survival ($p=0.039$). Therefore, due to subtype specificity, *PARP1* expression level and *TP53* mutation status are reliable marker candidates to distinguish *Proneural* and *Classical* subtypes, with prognostic and therapeutic implications in GBM.

Detection of *TP53* mutations besides *ATRX* mutations is a key factor in neuropathological diagnosis and also has prognostic and therapeutic relevance. The immunohistochemical detection of p53 protein is often used for the prediction of its mutation status, because *TP53* alterations can result in p53 accumulation in the nuclei of tumour cells. Despite controversy on the correlation between p53 accumulation and *TP53* mutational status, immunohistochemical detection of overexpressed protein has long been used as a surrogate method for mutation analysis. The additional aim of recent work was to characterise the IHC expression features of *TP53* somatic mutations and define their occurrence in cancer including gliomas. A large-scale database analysis was conducted in the IARC TP53 Database (R17). *TP53* mutations were divided into three IHC groups according to mutation frequency and IHC positivity. Among the IHC groups, significant correlations were observed with age at diagnosis

in breast, bladder, liver, haematopoietic system and head & neck cancers. An increased likelihood of false negative IHC associated with rare nonsense mutations was observed in certain tumour sites. Our study demonstrates that p53 immunopositivity largely correlates with *TP53* mutational status but expression is absent in certain mutation types.

7. New scientific results

- High PARP1 expression may play a role in the malignant transformation of the tumours, and its increased mRNA expression can be observed in high grade astrocytomas. In addition, *PARP1* copy numbers also correlate with higher histological grades.

- *PARP1* is also associated with the molecular heterogenity of tumours. Its increased expression correlates with the mutation status of *ATRX* and *TP53* genes. The immunohistochemical expression of PARP1 is also associated with IHC positive p53 and IHC negative ATRX cases.

- Among the clinically relevant subgroups of glioblastoma, *Classical* and *Proneural* GBMs exhibited increased *PARP1* mRNA expression level. Furthermore, patients with higher *PARP1* level have significantly shorter overall survival in the *Classical* subgroup.

- Detection of PARP1 expression serves as a diagnostic marker for GBM. Based on the genetic profile of the GBM subgroups, it is assumed that the *Classical* and the *Proneural* GBMs can be separated from the other two molecular subgroups according to *PARP1* and *TP53* mutation status.

- The majority of somatic *TP53* mutations (> 80%) exhibit positive p53 IHC staining in glial tumours. The *TP53* mutations are divided into three groups according to the frequency and IHC positivity, which correlates with the age of certain tumour types.

- Nonsense *TP53* mutations, which cannot be detected by immunohistochemistry, are rare in the glial brain tumours. On the other hand, nonsense *TP53* mutations are more frequent in certain tumour types (breast, lung, bladder and colorectum).

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List of publications related to the dissertation

1. **Murnyák, B.**, Kouhsari, M. C., Hershkovitch, R., Kálmán, B., Marko-Varga, G., Klekner, Á., Hortobágyi, T.: PARP1 expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma.
   Oncotarget. *8* (28), 46348-46362, 2017.
   DOI: http://dx.doi.org/10.18632/oncotarget.18013
   IF: 5.168 (2016)
2. **Murnyák, B.**, Hortobágyi, T.: Immunohistochemical correlates of TP53 somatic mutations in cancer.
   Oncotarget. *7* (40), 64910-64920, 2016.
   DOI: http://dx.doi.org/10.18632/oncotarget.11912
   IF: 5.168

List of other publications

3. Bencze, J., Simon, V., Bereczki, E., Majer, R., Varkoly, G., **Murnyák, B.**, Kálmán, J., Hortobágyi, T.: A Lewy-testes demencia klinikai és neuropatológiai jellemzői.
Orvosi Hetilap. 158 (17), 643-652, 2017.
DOI: http://dx.doi.org/10.1556/650.2017.30735
IF: 0.349 (2016)

4. Hortobágyi, T., Bencze, J., **Murnyák, B.**, Kouhsari, M. C., Bognár, L., Marko-Varga, G.: Pathophysiology of meningioma growth in pregnancy.
Open Med. 12, 195-200, 2017.
IF: 0.294 (2016)

5. Hortobágyi, T., Szabó, R., **Murnyák, B.**: The Theragnostic Relevance of DNA Methylation Analysis in Cancer.
Clin. Oncol. 2 (1289), 1-2, 2017.

6. Csonka, T., **Murnyák, B.**, Szepesi, R., Bencze, J., Bognár, L., Klekner, Á., Hortobágyi, T.: Assessment of candidate immunohistochemical prognostic markers of meningioma recurrence.
Folia Neuropathol. 54 (2), 114-126, 2016.
IF: 1.093

7. Szepesi, R., Csokonay, Á., **Murnyák, B.**, Kouhsari, M. C., Hofgárt, G., Csiba, L., Hortobágyi, T.: Haemorrhagic transformation in ischaemic stroke is more frequent than clinically suspected: a neuropathological study.
J. Neurol. Sci. 368, 4-10, 2016.
DOI: http://dx.doi.org/10.1016/j.jns.2016.06.055
IF: 2.295

8. **Murnyák, B.**, Szepesi, R., Hortobágyi, T.: A központi idegrendszert érintő családi daganatszindrómák molekuláris háttere.
Orvosi Hetilap. 156 (5), 171-177, 2015.
DOI: http://dx.doi.org/10.1556/OH.2015.30092
IF: 0.291

9. **Murnyák, B.**, Csonka, T., Hortobágyi, T.: A meningeomák molekuláris patológiája.
Ideggyogy. Szle. 68 (9-10), 292-300, 2015.
DOI: http://dx.doi.org/10.18071/isz.68.0292
IF: 0.376

10. **Murnyák, B.**, Bognár, L., Klekner, Á., Hortobágyi, T.: Epigenetics of Meningiomas.
Biomed Res. Int. 2015, 1-6, 2015.
DOI: http://dx.doi.org/10.1155/2015/532451
IF: 2.134

11. **Murnyák, B.**, Bodoki, L., Nagy-Vincze, M., Griger, Z., Csonka, T., Szepesi, R., Kurucz, A., Dankó, K., Hortobágyi, T.: Inclusion body myositis: pathomechanism and lessons from genetics.
Open Med. 10, 188-193, 2015.

12. Bodoki, L., Nagy-Vincze, M., Griger, Z., Csonka, T., **Murnyák, B.**, Kurucz, A., Dankó, K., Hortobágyi, T.: Inclusion body myositis - a case based clinicopathological update.
Cent. Eur. J. Med. 9 (1), 80-85, 2014.
IF: 0.153

13. Csonka, T., **Murnyák, B.**, Szepesi, R., Kurucz, A., Klekner, Á., Hortobágyi, T.: Poly(ADP-ribose) polymerase-1 (PARP1) and p53 labelling index correlates with tumour grade in meningiomas.
Folia Neuropathol. 52 (2), 111-120, 2014.
DOI: http://dx.doi.org/10.5114/fn.2014.43782
IF: 1.568

14. **Murnyák, B.**, Csonka, T., Klekner, Á., Hortobágyi, T.: Alacsony grádusú glialis daganatok előfordulása és molekuláris patológiája.
Ideggyogy. Szle. 66 (9-10), 305-311, 2013.
IF: 0.343

15. **Murnyák, B.**, Csonka, T., Hegyi, K., Méhes, G., Klekner, Á., Hortobágyi, T.: Magas grádusú gliomák előfordulása és molekuláris patológiája.
Ideggyogy. Szle. 66 (9-10), 312-321, 2013.
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