**Angelika Stapińska-Syniec**

# The correlation between DNA hydroxymethylation and WHO grade of glial tumors of the central nervous system and its clinical implications.

## Summary

Keywords: 5-hydroxymethylcytosine, TET enzymes, glioma, glioblastoma, DNA hydroxymethylation

Introduction:

Gliomas are the most common primary brain and spinal cord tumors constituting 40% of primary intracranial tumors and 70-80% of primary malignant central nervous system tumors. Glioblastoma is responsible for almost half of cases of primary malignant central nervous system tumors. Contrary to previous editions, the 2016 World Health Organization (WHO) classification of nervous system tumors, as same as the latest 2021 edition, are focused on gliomas classification depending on molecular and genetic markers in connection with histopathology. The epigenetic analysis is becoming a crucial aspect of diagnostics and treatment of many tumors. So far, the role of DNA methylation and hydroxymethylation in different tumors has been studied in terms of gene expression regulation during carcinogenesis and its possible clinical implications.

Aims and objectives of doctoral thesis:

The aim of the study is an attempt to correlate the DNA hydroxymethylation and ten-eleven translocation (TET) enzymes gene expression with the WHO grade of gliomas and its clinical implications.

Material and methods:

The study group constitutes 40 patients with brain tumors who were operated on at the Department of Neurosurgery, Institute of Psychiatry and Neurology, in a period from 2018 to 2020. A sample of brain tumor was collected from every patient for standard histopathological evaluation, as well as another sample of brain tumor and blood were collected to perform molecular analysis of DNA hydroxymethylation and TET gene expression. Genetic analyses of 40 tumor samples and 40 blood samples were performed at the Department of Clinical Cytology, Centre of Postgraduate Medical Education. 5-hydroxymethylcytosine (5-hmC) levels in DNA isolated from tumor samples were evaluated by EpiJET Kits. Also, quantitative real-time PCR of TET1, TET2, and TET3 genes mRNA comprised in tumors and blood samples were done. The retrospective analysis of clinical data was performed based on the patient's medical history at the Department of Neurosurgery and Neurosurgical Out-patient Clinic.

Results:

The analysis of 5-hmC levels in the study group revealed the differences in the 5-hmC levels depending on the central nervous system (CNS) tumor WHO grade. The 5-hmC levels were lower with the higher WHO grade, except for the subpopulation of patients with tumors in the 4 grade with high 5-hmC levels. Tumor TET1, TET2, and TET3 genes expression measured by mRNA levels was decreased with the higher WHO grade, however, TET enzymes activity in blood did not reveal any statistically significant differences depending on the tumor WHO grade. The correlation analysis performed for molecular and clinical parameters revealed tumor TET1 and TET2 genes expression as independent factors, that are statistically significant

negatively correlated with patients' survival. The correlation between 5-hmC levels and TET1, TET2, and TET3 genes expression did not reveal any statistical significance in both, tumors, and blood. Multiparametric analysis of molecular parameters indicated only 5-hmC as a factor statistically significantly connected with the patients' survival (p=0,05), however, the 5-hmC level did not affect the chance of survival significantly (OR 1,007; CI 95%; 0,9004-1,214).

Conclusions:

The study revealed the decrease in 5-hmC levels and tumor TET1, TET2, and TET3 genes expression with the higher WHO grade, except for the subpopulation of patients with tumors in the 4 grade with high 5-hmC levels. Significant connection of the 5-hmC level on patient`s survival, however without significant imprint on the chance of survival has been confirmed. Moreover, the correlations between the 5-hmC level and both, tumor, and blood TET1, TET2, and TET3 genes expression did not reveal any statistical significance, which deprives this parameter as a possible tumor biomarker. However, straightforward verification of 5-hmC and TET utility as possible biomarkers requires more studies on a larger population of patients.