

THE STUDY OF M6A EPIMODIFICATION REGULATING GENES EXPRESSION AND M6A EPIMARK LEVEL IN HUMAN ASTROCYTOMAS

Katažyna Samaite¹, Arimantas Tamašauskas¹, Giedrius Steponaitis¹

¹ Laboratory of Molecular Neurooncology, Neuroscience Institute, Lithuanian University of Health Sciences
Eivenių st. 2, Kaunas LT-50161, Lithuania
katazynasamaite@gmail.com

Astrocytomas are astrocytes derived, diffusely infiltrating and the most frequent primary tumors in human brain. Astrocytomas according to tumor malignancy are classified into 4 grades of which the most common and aggressive are 4th grade tumors called glioblastomas (GBM) [1]. Glioblastoma is a life-threatening brain tumor showing a median overall patient survival between 10 and 20 months after the resection. The incidence of glioblastoma is approximately 3-4/100,000, increases with age, and peaks in patients aged 50-60 years [2]. Epitranscriptome, in general can be understood as a chemical modifications of RNA bases, is recently discovered as an additional regulation level of biological information. More than 170 chemical modifications types of RNA are known up to date. Abundant levels of RNA modification are found in tRNA and rRNA, and slightly lower levels in mRNA and ncRNA [3, 4]. Methylation of adenosine at N6 position- m6A is identified as the most frequent modification in mRNA, microRNA and lncRNA and is also associated with cancer, obesity and infertility [5, 6]. m6A modification is controlled by three genes types: methyltransferases- „writers“ (METTL3, METTL14, WTAP), demethylases- „erasers“ (ALKBH5, FTO) and „readers“ (YTHDC1/2, YTHDF1/2/3) [7].

The aim of this research was to evaluate the significance of m6A epi-modification level and m6A regulating genes expression for astrocytoma malignancy, patient age and survival. The study enrolled n= 58 specimens of II and IV grade human astrocytomas (14 and 44, respectively). RNA from tumors specimens was purified applying TRIzol method following tissues cryo-homogenization. m6A modification detection was made applying fluorometric ELISA method using “EpiQuik™ m6A RNA Methylation Quantification Kit” according to the manufacturer’s recommendations. Gene expression of two m6A writers and two erasers (METTL3, WTAP and ALKBH5, FTO) were analyzed applying RT-qPCR. Results were evaluated by ΔCt method, GraphPad Prism software used for statistical calculations. Results showed that the expression of METTL3 (p<0.0001), ALKBH5 (p=0.0005) and FTO (p<0.0001) genes was associated with astrocytoma malignancy, patients survival (METTL3, FTO (p<0.01)) and IDH status (METTL3 (p<0.0001), ALKBH5 (p=0.0005), FTO (p<0.0001)). m6A modification level of total tumor specimens RNA did not showed any significant differences between tumor grade and patient clinical data as survival, patient age as well as IDH status. Such a data might be a result of ribosomal RNA (rRNA) which represents more than 95% of the total RNA, thus further investigation is essential to clarify the significance of N6-methyladenosine modification level in mRNA and ncRNA for astrocytomas.

To conclude, mRNA expression analysis of m6A writers and erasers revealed that METTL3, ALKBH5, FTO could serve as potential targets for astrocytoma diagnostics and prognostics.

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