SEMA3A, SEMA3C, SEMA3F AND *NRP1* GENES EXPRESSION ANALYSIS IN DIFFERENT GRADE ASTROCYTIC GLIOMA TUMORS

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Glioma is the most common tumor in the central nervous system. The most aggressive form of glioma is glioblastoma (grade IV), which is characterized by increased cell proliferation, invasion and formation of new blood vessels around the tumor. Despite the complex treatment of glioblastoma (surgery, chemotherapy and radiation therapy), the survival time of patients is generally short (approximately 15 months) [1]. Therefore, scientists try to find a way to inhibit tumor development and identify potential biomarkers for an earlier glioma prognosis and also to discover tumor-specific molecules that could be used in target treatment therapies or which could help to predict the response to treatment. According to the latest studies, secreted class 3 semaphorins (Sema3) and their receptors play an important role in regulating cell viability, invasiveness, and angiogenesis in tumorous diseases [2-4]. However, not all SEMA3 proteins have the same effect on tumor diseases because, for example, increased SEMA3A expression in oral cancer cells significantly reduces tumor growth [5], increased SEMA3F expression inhibits cell invasiveness in breast cancer and melanoma [6] but increased SEMA3C expression promotes tumor progression in prostate, liver, stomach cancer and is associated with shorter patient lifespan [7]. Therefore, the aim of this study was to analyze the changes of *SEMA3A*, *SEMA3C*, *SEMA3F*, and *NRP1* gene expression in different grade astrocytic glioma tumors and to identify the association between gene expression and patients' clinical data.

To achieve this goal, total RNA was extracted from frozen tumor tissue and cDNA was synthesized by using reverse transcriptase. Before analyzing gene expression, *SEMA3A*, *SEMA3C*, *SEMA3F*, and *NRP1* gene quantitative real-time PCR (qRT-PCR) conditions were optimized by gradient PCR and the expression of these genes was determined by qRT–PCR analysis with SYBR Green fluorescent dye. During the study, beta-actin gene (ACTB) was selected for internal control and gene expression changes were compared to healthy brain tissue. The comparative $2^{-\Delta\Delta Ct}$ method was used for the calculations of genes mRNA expression. The obtained data was used to investigate associations between *SEMA3A*, *SEMA3C*, *SEMA3F*, and *NRP1* gene expression and the patients' clinical data (gender, age before surgery, tumor grade, and survival of patients). Also, the correlation between expression of *SEMA3A*, *SEMA3C*, *SEMA3F* and their receptor *NRP1* in gliomas was evaluated.

69 tumor samples taken from patients with the diagnosis of I – IV grade astrocytic glioma tumors were analyzed. Increased *NRP1* and *SEMA3F* gene expressions were observed in higher grade (III-IV) gliomas as compared with lower grade gliomas (*Mann-Whitney* test, $p = 0.001$ and $p = 0.01$, respectively). In contrast, increased *SEMA3C* gene expression was observed in lower grade (I-II) gliomas (*Mann-Whitney* test, $p < 0.05$). The upregulation of *SEMA3A* was associated with poor patient prognosis in higher grade astrocytoma (*Kaplan-Meier* test, $p < 0.01$). Increased *SEMA3F*, *SEMA3A* and *NRP1* gene expressions were observed in older patients (*Mann-Whitney* test, $p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively). In addition, the correlation between *SEMA3F* and *NRP1* expressions was found (*Spearman's rank correlation coefficient*, $r_s = 0{,}644$ and $p < 0.001$). For all studied genes (*SEMA3A*, *SEMA3C*, *SEMA3F* and *NRP1*) statistical difference between genders was not observed. These findings suggest that *NRP1* and *SEMA3F* genes could be used as a prognostic biomarkers for determining the malignancy grade of glioma, whereas changes in *SEMA3A* and *SEMA3C* gene expression could be used to predict the survival of patients.

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