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BRIEF REPORT

ENTPD1 (CD39) and NT5E (CD73) expression in human glioblastoma: an in silico analysis

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Abstract

Glioblastoma (GB) is the most common primary brain tumor in adults and carries a dismal prognosis, despite the best available treatment. The 2021 WHO Classification of CNS tumors incorporated molecular profiling to better define the characteristics and prognosis of tumor types and subtypes. These recent advances in diagnosis have not yet resulted in breakthrough therapies capable of shifting the treatment paradigm. NT5E/CD73 is a cell surface enzyme that participates in a complex purinergic pathway in synergy with ENTPD1/CD39 producing extracellular adenosine (ADO) from ATP. ADO promotes tumor progression by inducing immunosuppression, stimulating adhesion, invasion, and angiogenesis. In this study, we performed an in silico analysis of 156 human glioblastoma samples in an unexplored public database to investigate the transcriptional levels of NT5E and ENTPD1. The analysis revealed a significant increase in transcription levels of the genes under study in GB samples versus non-tumor brain tissue samples, in concordance with previous studies. High transcriptional levels of NT5E or ENTPD1 were independently related to a decrease in overall survival ($p = 5.4e\text{-}04$; 1.1e-05), irrespective of the IDH mutation status. NT5E transcriptional levels were significantly higher in GB IDH wild-type patients compared to GB IDH-mutant; however, ENTPD1 levels showed no significant difference, $p \leq 0.001$. This in silico study indicates the need for a deeper understanding of the purinergic pathway relation to GB development, also inspiring future population studies that could explore ENTPD1 and NT5E not only as prognostic markers but also as potential therapeutic targets.

Keywords Glioblastoma · In silico · ENTPD1 · NT5E · CD39 · CD73

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Abbreviations

ATP	Adenosine triphosphate
ADO	Adenosine
CNS	Central nervous system
GB	Glioblastoma
IDH	Isocitrate dehydrogenase
NK	Natural killer
OS	Overall survival
WHO	World Health Organization
WT	Wild type

Introduction

Glioblastoma (GB) is the most common primary brain tumor in adults, with an incidence of 3.19:100,000 inhabitants in the USA, affecting mainly the elderly population [1]. The average survival time can vary from 3 months (if no treatment is received) to up to 16 months on average if the patient undergoes maximum safe resection, radiotherapy, and chemotherapy [1, 2]. Virtually, all patients relapse after 10 months and only about 17.5% of patients survive after the 2nd year of diagnosis [1, 2]. There is also a devastating impact on the quality of life associated with progressive neurological symptoms and adverse effects from treating this tumor.

As a grade IV glioma, atypia, mitosis, necrosis, and vascular infiltration define its heterogeneous histology. However, grouping gliomas into molecular types has been used modernly and, according to Gravendeel et al. [3], genotypes are more accurate predictors of survival than histology, assuming great importance in clinical decisions. The 2016 World Health Organization (WHO) Classification for Central Nervous System (CNS) tumors incorporated the isocitrate dehydrogenase (IDH) gene mutation [4] which is associated with younger age presentation and longer survival, in contrast to patients with wild-type IDH gene (wild type or WT) [5]. The 2021 WHO Classification of Tumors of the CNS eliminated the term "Glioblastoma, IDH-mutant" and replaced it with the term "Astrocytoma, IDH-mutant" that covers grades 2 to 4, and also included the newly recognized Diffuse Astrocytoma, MYB- or MYBL1-altered [6]. However, in the studied population, patients were grouped according to the 2016 WHO Classification; hence, we adopted that classification for this study. Thus, both diffuse astrocytomas grade 2 and anaplastic astrocytoma grade 3 with IDH WT status are not included in this analysis, while astrocytoma grade 4 is represented as GB IDH-mutant [3].

CD73 is a cell surface enzyme encoded by the NT5E gene that participates in the purinergic pathway in synergy with CD39, encoded by the ENTPD1 gene, promoting the hydrolysis of extracellular adenosine triphosphate (ATP) into adenosine (ADO) [7]. In the tumor microenvironment, ADO promotes tumor progression not only by stimulating adhesion, proliferation, invasion, and angiogenesis but also by inducing immunosuppression of tumor-associated immune cells, mainly by stimulating its A2b receptor. This specific receptor is also associated with the glioma chemoresistance profile [8, 9]. Both CD73 and CD39 are overexpressed in GB tumor tissue [8, 10, 11].

The prognostic and therapeutic potential of these genes has been explored in contemporary literature; however, it has not yet been incorporated into treatment [8, 12]. Translational experimental studies have shown that CD39 and CD73 silencing is associated with decreased tumor growth [13, 14]. A recent in silico analysis explored the relationship between CD73, GB, and intratumoral natural killer (NK) cell infiltration. This study showed that the degree of expression of CD73 is inversely related to disease-free survival time. Patients with high expression of both CD73 and CD39 genes had a shorter survival than others. The survival time between patients with high expression of only one of these genes and patients with low expression of both genes was similar [11].

Based on this, we asked whether the degree of independent expression of NT5E/CD73 and ENTPD1/CD39 is associated with a shorter survival time in patients diagnosed with GB. To investigate the prognostic value and consequent potential therapeutic target of CD73 and CD39 in this disease, we conducted an in silico analysis of 156 representative tumor samples in unexplored public databases [3]. We then generated Kaplan–Meier curves for survival time in samples with high and low expression of these genes. Therefore, the study aims to evaluate the expression of CD73 and CD39 mRNA comparing tumor and non-tumor tissue from the samples and to correlate with the survival time of patients diagnosed with GB from a public dataset.

Methodology

The mRNA expression of target genes was normalized in all samples within R2: Genomic Analysis and Visualization Platform (http://r2.amc.nl) transformed from signal intensity. The classification of subgroups and the number of patients in each subgroup was performed according to the availability of stratification in the database (French — https://hgserver1.amc.nl/cgi-bin/r2/main.cgi). The analysis separates the samples from the dataset into high and low gene expression. Each expression value was ordered ascendingly as a cut-off point to form two groups and test the *p*-value in a log rank test. The test will find the maximum significant expression cut-off point for the survival analysis. Therefore, we found the best possible Kaplan–Meier curve by the log rank test. All subgroups were compared using a Kruskal–Wallis test for significance and the false discovery rate method, followed by the post hoc Welch *t* test performed using the R2 platform. For gene expression analyses, $p \leq 0.001$ values were considered statistically significant. The study was exploratory.

Overall survival (OS) was measured from date of initial diagnosis to death or date of last follow-up, using OS combined with gene expression data according to the availability of each database. Survival distribution was estimated according to the Kaplan–Meier method using a median cutoff and log rank statistics; $p \leq 0.05$ was considered statistically significant.

An institutional ethical approval was not required for this study, and it was not pre-registered.

No sample calculation was performed, because all available data that could be used were used.

Results

In silico analysis of the dataset of samples from 156 GB patients revealed a significantly higher expression of NT5E and ENTPD1 when compared to normal brain tissue or non-tumor brain tissue (control), $p \leq 0.001$.

Kaplan–Meier curve analysis showed that high NT5E or ENTPD1 expression in GB samples is significantly associated with decreased OS ($p = 5.4e\text{-}04$, 1.1e-05). $p \leq 0.05$ was considered significant.

Transcriptional levels of target genes were also evaluated in samples from GB patients carrying the r132 IDH mutation ($n = 33$), compared to WT IDH ($n = 95$). NT5E levels are significantly higher in patients with GB IDH WT compared to the mutated GB IDH, while ENTPD1 levels have not shown significant differences between samples. $p \leq 0.001$was considered significant.

Patients with GB IDH mutated and higher transcriptional levels of NT5E or ENTPD1 had significantly shorter OS than patients with GB IDH mutated and lower transcriptional levels of NT5E or ENTPD1 ($n = 33$; $p = 0.011$; $p = 3.2e\text{-}03$). Patients with GB IDH WT and increased NT5E or ENTPD1 also had significantly shorter OS than the other patients ($n = 92$; $p = 0.031$; $p = 2.5e\text{-}03$). $p \leq 0.05$ was considered significant.

Discussion

CD39 is an enzyme expressed on the cell surface that hydrolyzes extracellular ATP to AMP, which can later be converted to ADO by the action of CD73, thus working together in the adenosinergic cascade. Extracellular ATP is a pro-inflammatory metabolite that binds to P2X receptors on T cells and induces cytokine production. Therefore, its hydrolysis by CD39 compromises the functionality of effector T cells and favors the increase in regulatory T cells. This inappropriate activation of T cells results in dysfunctional $CD8^+$ T cell states, including anergy, exhaustion, and senescence [15].

CD73 is a regulator of glioma oncogenesis: on the one hand, its expression favors the growth, migration, and invasion of GB cells by producing ADO; on the other hand, its "downregulation" reduces the viability of gliomas and enhances the effect of temozolomide [13]. High NT5E expression is a prognostic predictor of lower OS in patients with the GB mesenchymal subtype [11]. Zhi et al. [16] suggest that in addition to adenosine formation, CD73 promotes the invasive and metastatic behavior of several cancers by regulating the interaction of cancer cells with the extracellular matrix.

Enzymes of the IDH group catalyze the oxidative decarboxylation of isocitrate and, therefore, play important roles in cell homeostasis. The presence of the mutated IDH predicts a median survival of 31 months, whereas patients with IDH WT have a median survival of 15 months [4]. We found that IDH WT and high transcriptional levels of ENTPD1 or NT5E are independent predictors of poor prognosis, which could result in a different tumor subtype.

This in silico analysis corroborated data in the literature that NT5E and ENTPD1 are overexpressed in GB when compared to normal brain tissue [8, 10, 11]. Furthermore, it also found that a higher transcription level of these genes in tumor tissue is associated with a shorter OS when compared to the expression levels of these genes among patients with GB. This finding reinforces the importance of high expression of both genes as predictors of prognosis and points to the need for further studies that seek to analyze the correlation of expression of these genes with the prognosis of patients with GB.

We also found that patients with GB IDH WT have higher transcriptional levels of NT5E but not ENTPD1 when compared to patients with the GB IDH r132 mutation. An alternative explanation for these findings would be the formation of ADO via the non-canonical pathway mediated by CD38. While CD38 is not directly involved in the synthesis of adenosine, it can indirectly contribute to adenosine production by generating ADPR and cADPR, which can be further metabolized into adenosine by CD73 [17]. Since there is no significant difference in the transcription of ENTPD1 in patients with GB IDH WT and GB mutated IDH, it can be hypothesized that the most important pathway for adenosine production is not the canonical pathway, mediated by CD39, but the non-canonical axis mediated by CD38 (an NAD+ nucleosidase), the ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1, also known as CD203a or PC-1) [18]. Blatcher et al. [19] demonstrated that by inhibiting CD38, one can significantly inhibit the progression of gliomas in rats and prolong their survival.

Furthermore, we found that high expression of both NT5E and ENTPD1 is related to decreased OS regardless of IDH status. These data were not previously described and may contribute to the understanding of the worst prognosis

of these patients. This finding reinforces the importance of the expression of CD73 and CD39 in tumorigenesis and, therefore, as prognostic markers and potential targets for the development of new treatments.

Conclusion

GB carries a poor prognosis and the available lines of treatment have barely improved patient survival in the last decades. There is growing evidence that CD73 promotes GB patogenesis, either through canonical or non-canonical adenosine pathways. The present study corroborates data already present in the literature and further explores genetic markers in the purinergic pathway.

GB IDH WT patients have a worse prognosis than GB IDH-mutant patients and express higher levels of CD73 but not CD39 in their tumor samples. This could be investigated in future studies by concomitant evaluation of genes present in the canonical and non-canonical adenosine pathways in order to elucidate which adenosine pathway plays a bigger role in GB tumorigenesis. The degree of expression of CD73 and CD39 is associated with a shorter median survival time in patients diagnosed with GB, regardless of IDH status.

These data indicate the need to carry out more population studies that explore the expression of CD73 and CD39 not only as prognostic markers but also as potential therapeutic targets.

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Authors' contribution MAS and EB contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by MAS and EB. All authors contributed to writing this first draft of the manuscript. All authors read and approved the final manuscript.

Data availability The data can be obtained upon request to the corresponding author or in https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?open_page=login.

Compliance with ethical standards

Conflicts of interest The authors declare no competing interests.

Ethical approval This work does not involve animals or human subjects.

Consent for publication All the authors provided their consent for the publication of this study.

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